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COMPARATIVE STUDY OF THE APPLE ANTHRACNOSE AND PERENNIAL CANKER FUNGI [1]

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INTRODUCTION

The closely related apple-tree cankers, apple anthracnose, caused by *Neofabraea malicorticis* (Cordley) Jackson, and perennial canker, caused by *Gloeosporium perennans* Zeller and Childs, are native to the Pacific Northwest. The geographic ranges of the two overlap to some extent, but generally in the districts where one is abundant the other is either absent or rare.

A study of these two diseases and their causal agents during the past 6 years has shown them to be similar in so many respects that identification has sometimes been uncertain or impossible. The fungi, as described from their typical development, are quite distinct, but in the regions where both occur the definite identification of intermediate types becomes a difficult problem.

Previous studies have been devoted almost entirely to practical methods of control, and the etiological phases have been greatly neglected. Since a preliminary study of the previously unreported ascigerous form of the perennial canker organism had shown it to be very similar to *Neofabraea malicorticis*, a comparative study of the two fungi was undertaken and the results are reported in this paper.

LITERATURE REVIEW [3]

The literature of apple anthracnose has been summarized to recent date in books by Heald (*20, pp. 500–511*)[4] and by Owens (*33, pp. 288–299*). The reader is referred to these standard texts for detailed historical information.

The apple anthracnose fungus (*Neofabraea malicorticis*) first attracted attention as a serious orchard parasite about 1891. Early investigations (*7, 9*) proved the disease to be due to a parasitic fungus. The imperfect or acervular stage of this organism was described from Oregon by Cordley (*7, 8, 9*) as *Gloeosporium malicorticis*, and at about the same time by Peck (*34, p. 21*) as *Macrophoma curvispora* from specimens submitted to him from British Columbia. The former determination was confirmed by Lawrence (*27*), although in a later article (*28, pp. 32–33*) he used the name *Myxosporium curvisporum*

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[2] Special appreciation is expressed to Dr. John W. Roberts, of this Division, and to Dr. S. M. Zeller, of Oregon State College, under whose supervision the work was carried on. Leroy Childs, superintendent of the Hood River branch station of the Oregon Agricultural Experiment Station; Dr. H. R. McLarty, of the Dominion Experimental Farms, Summerland, British Columbia; and E. L. Reeves, of this Division, gave helpful suggestions during the work. Other investigators, both local and Canadian, were helpful in various ways. The writer is grateful for the help received from these workers.

[3] A complete bibliography of the literature dealing with the apple anthracnose and perennial canker diseases appears in the author's thesis. See footnote 1.

[4] Italic numbers in parentheses refer to Literature Cited, p. 663.

(Peck) Sacc. The ascigerous stage was discovered by Jackson (*24*) in 1909, for which he established the new genus *Neofabraea* and named *N. malicorticis* (Cordley) Jackson as the type species.[5]

A similar canker disease was distinguished by Zeller and Childs (*41*, *42*) in 1925, although it was undoubtedly present in the Pacific Northwest long before this discovery (*14*). It was found that this "false anthracnose" was not controlled by the copper sprays effective against the common apple anthracnose. The disease was termed "perennial canker" because of the apparently perennial character of the cankers, and the causal organism was described as *Gloeosporium perennans* Zeller and Childs (*42*). Infections occurred through wounds of various kinds, whereas apple anthracnose resulted from infections through apparently sound bark. The perennial canker fungus produced straight to slightly curved rather than curved or hooked conidia, and showed less diastatic power on certain starchy media. McLarty (*29*) has reviewed the perennial canker literature and has presented the facts as they are commonly accepted at the present time.

Miller (*30*) gives results of a special comparative study with these fungi. He states that in his tests both organisms reacted in a similar way. Although certain differences were apparent with different cultures, more variation existed between the various strains of either species than between the species themselves. Introduction of tannic acid in the culture medium, however, appeared to inhibit the average growth rates of all strains of *Neofabraea malicorticis* to a greater degree than those of *Gloeosporium perennans*. Similar results were reported in a brief abstract by the writer (*25*), when malachite green was used as the inhibitory agent.

In previous work several characters have been reported by which one supposedly should be able to distinguish apple anthracnose from perennial canker (fig. 1, *A* and *B*). The most important of these are as follows:

(1) Anthracnose usually occurs west of the Cascade Range; perennial canker is most common east of this mountain range (*6*, *21*, *pp. 13–17*).

(2) Copper fungicides applied before fall rains prevent anthracnose infections but have little influence upon perennial canker [6] (*5*, *13*, *14*, *36*).

(3) The apple anthracnose fungus infects apparently sound bark, often through lenticels, and outbreaks are independent of the woolly apple aphid and low temperatures (*5*, *29*). Perennial canker infections occur only through evident injuries to the host. Galls produced on callus tissue by the woolly apple aphid (*Eriosoma lanigerum* (Hausmann)), which rupture at low temperatures, form the usual infection courts under natural conditions.

(4) Conidia from anthracnose cankers vary in shape from curved to "hooked"; those of perennial canker vary from straight to slightly curved. Lawrence (*27*) and Zeller and Childs (*42*) report that anthracnose conidia from apple fruit rots and in culture show curvature, whereas perennial canker conidia grown in the same way are relatively straight.

[5] Nannfeldt (*31*) transferred the genus *Neofabraea* to *Pezicula*, based upon the European species *N. corticola* (Edg.) Jorg., a species typical of the genus *Pezicula*. Drs. J. Walton Groves and G. E. Thompson have compared the genera and found them to be distinct. A notice of the error appears in a recent paper by Thompson (*40*).

[6] Barss, H. P. Northwestern apple tree anthracnose canker and fruit rot (Neofabraea malicorticis). Oreg. Agr. Col. Ext. Cir. 220, 3 pp. 1925. [Mimeographed.]

(5) The anthracnose fungus shows a greater diastatic activity on certain starchy media than the perennial canker organism (*42*).

(6) A perfect stage has been previously reported only for the anthracnose fungus (*24*).

(7) Fruit rots caused by *Neofabraea malicorticis* tend to show slightly more zoning or more concentrically ringed effects than fruit rots caused by *Gloeosporium perennans*, and minor differences appear in sporulation characters (*11*, *12*, *13*, *42*).

(8) Observations indicate that old anthracnose cankers tend to present a "fiddle-string" appearance, presumably because of failure of

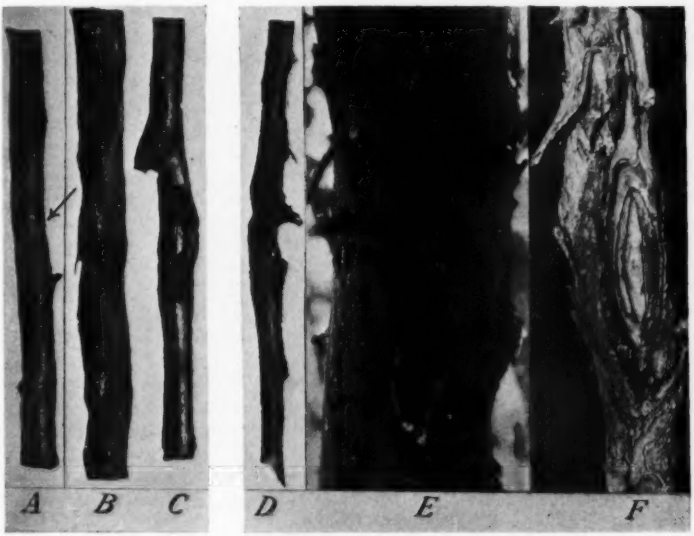

FIGURE 1.—Common sequence of the apple anthracnose and perennial canker diseases in orchards. *A*, *B*, *C*, Apple anthracnose: *A*, Primary infections through apparently sound bark, indicated by arrow; *B*, numerous infections developing below an old canker; *C*, twig showing eventual healing of a wound, which takes place unless the limb is girdled or callus tissue becomes injured and reinfected with perennial canker. *D*, *E*, *F*, Perennial canker: *D*, Primary infections through wounds; *E*, callus tissue injured by feeding of the woolly apple aphid, thus forming galls that rupture at low temperatures and become infected; *F*, perennial types of cankers resulting from annual reinfections of injured callus tissue.

the fungus to attack the bast fibers. Perennial cankers rarely show these exposed fibers.

DISTRIBUTION AND OCCURRENCE

The comparative distribution of the apple anthracnose and perennial canker fungi, based upon a survey of the literature, examinations of cankered specimens, and letters from workers in the various districts, is illustrated in figure 2.

Anthracnose seems, as a rule, to be peculiar to those humid regions of the Pacific Northwest that have a moderately high rainfall and mild winter temperatures (*6*). In general, this includes the humid division

of the Transition life zone (*1*), lying mostly west of the Cascade Range and having an average annual precipitation of approximately 35 to 80 inches. The disease is comparatively rare in northern California, common in western Oregon, western Washington, and British Columbia, and probably reaches its northern limit near Prince Rupert, British Columbia. A single report as far east as Nebraska was given by Heald (*20*). Hilborn [7] very recently reported the presence

FIGURE 2.—Comparative distribution of the apple anthracnose and perennial canker diseases in the Pacific Northwest.

of anthracnose in Maine. The anthracnose organism has been reported as occurring on pear and apple trees in Denmark (*16*) and Netherlands (*35*).

Perennial canker occurs most often east of the Cascade Range in the drier Transition and Sonoran zones, characterized by lower winter temperatures and usually with less than 25 inches of annual precipitation. Its range extends from southern Oregon to the northern end of the Okanagan Valley in British Columbia and from the Cascade Range to western Montana. Brien (*3, 4*) reported this canker organism as causing a fruit rot in New Zealand.

In the White Salmon-Hood River Valley area of Washington and Oregon, anthracnose considerably overlaps the range of perennial canker. The Columbia River breaks through the Cascade Range at this point, and climatic conditions are intermediate between those of

[7] HILBORN, M. T. NORTHWESTERN APPLE TREE ANTHRACNOSE FOUND IN MAINE. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 354. 1938. [Mimeographed.]

the humid coastal region and the semiarid interior. A similar condition occurs in the Okanagan Valley of British Columbia. There is an overlapping of the diseases near Armstrong, where the precipitation is greater than that of the lower Okanagan Valley, in which perennial canker is dominant. Occasionally there are reports of the finding of anthracnose cankers in perennial canker areas. It is to be noted that such specimens are generally found in the mountainous foothill sections where precipitation increases and other climatic factors are different from those of the bordering perennial canker area. The rare appearance of perennial canker in regions where anthracnose is of common occurrence appears to be correlated with unusually severe winter temperatures.

An examination of the conidia of *Neofabraea malicorticis* produced in cankers from the above areas impresses one with the variation in spore shape exhibited by the fungus. Conidia from the occasional anthracnose cankers found east of the Cascade Range, or those from the intermediate areas, often appeared identical with, or even less curved than, the *Gloeosporium perennans* conidia in the more humid sections. Conidia in perennial cankers from the dry sections were practically straight, whereas considerable curvature might be present in those from the overlapping range. A somewhat progressive increase in degree of curvature of the conidia appeared to occur from the eastern to the western limits, so that in the strictly coastal sections anthracnose conidia were decidedly curved or "hooked." While this general trend was apparent, exceptions were frequent. Besides this regional variation, conidial shapes of known strains varied somewhat from season to season. The difficulty often encountered in identifying the two organisms from the size or shape of the conidia is apparent in figure 3, where typical conidia are illustrated in comparison with some intermediate regional forms and with the usual types of conidia produced in cultures and on apple rot tissue under Hood River conditions. Host variety seemed to have less influence on conidial curvature than did type of tissue infected or local environmental conditions.

HOST RELATIONS

The anthracnose and perennial canker fungi attack similar host plants (*19*, *25*, *36*, *41*). The former infects young trees and bark more commonly than well-matured trees, and in general, early-maturing varieties of apples are less susceptible than fall sorts. However, very few definite data are available concerning varietal resistance. Resistance to the perennial canker disease among apple varieties corresponds to their winter hardiness and freedom from woolly aphids. Individuals of a single variety may vary in these respects according to their vigor or because of other complex factors. *Gloeosporium perennans* was reported to have been isolated from cherry buds dying during the winter in Oregon (*32*), but neither fungus is commonly encountered on stone-fruit trees.

Shear and Cooley (*38*) showed by inoculations that trees have a definite period of susceptibility to the perennial canker organism. Susceptibility increased rapidly from a low point during September, reached a maximum in November and December, and subsided to infrequent infections by the middle of January. McLarty (*29*) proved that without freezing injury to the tissue the fungus was unable to

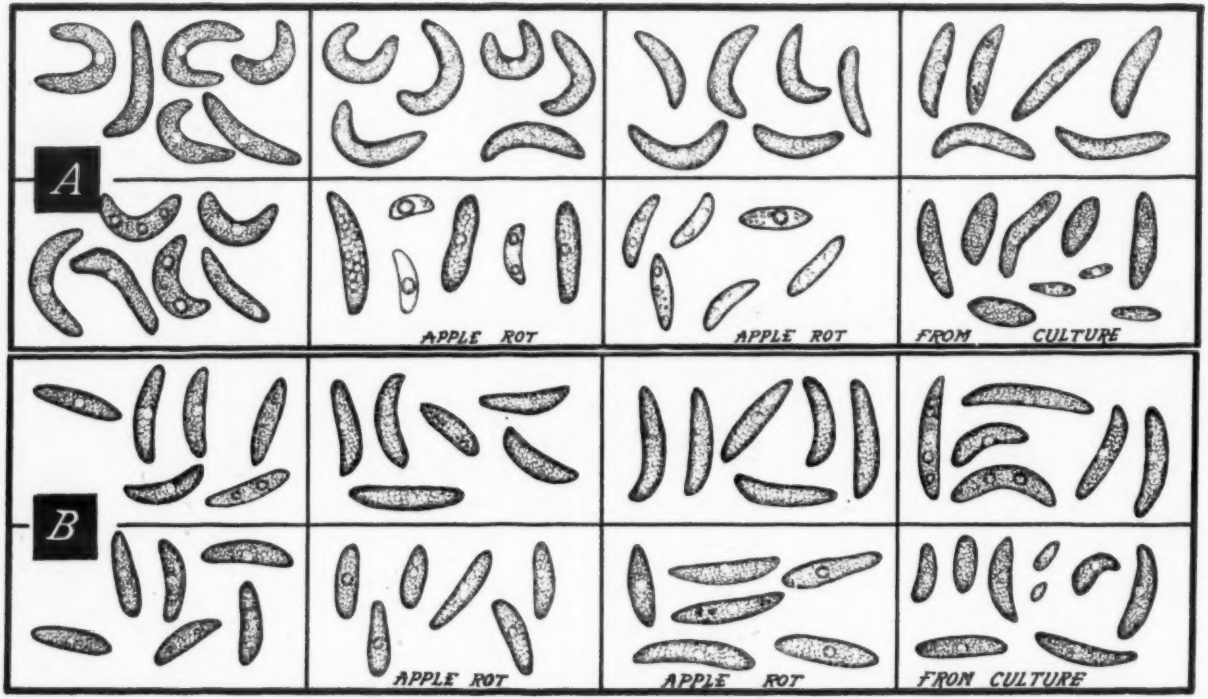

FIGURE 3.—Variation in conidial shapes of *Neofabraea malicorticis* and *Gloeosporium perennans*: *A*, *N. malicorticis*; *B*, *G. perennans*. Typical conidia are shown at the extreme left in each section, and intermediate forms from the overlapping geographical ranges are shown to the right. Types formed on apple rot tissue and in culture at Hood River are indicated. Approximately ×450.

infect the host, even when it was inoculated into the tree during the susceptible period. The detailed behavior of the anthracnose fungus in relation to periods of host susceptiblity has not been reported, but it appears to be similar to perennial canker.

It was thought possible that parallel inoculations with anthracnose and perennial canker fungi into various host species might yield some distinctive characters of taxonomic value. Accordingly, 1 typical strain of each fungus was inoculated into 12 genera of the Rosaceae, considered in its broadest sense, and into 30 species in 10 other families. Cankers developed from inoculations with each fungus on the following rosaceous hosts: Apple (*Malus sylvestris* Mill.), Siberian crab (*M. baccata* Borkh.), Oregon crab (*M. rivularis* Roemer), pear (*Pyrus communis* L.), quince (*Cydonia oblonga* Mill.), peach (*Amygdalus persica* L.), serviceberry (*Amelanchier pallida* Gr.), apricot (*Prunus armeniaca* L.), plum (*Prunus* sp.), cherry (*P. avium* L.), pin cherry (*P. emarginata* Walp.), flowering quince (*Chaenomeles* sp.), hawthorn (*Crataegus* sp.), and the wild and cultivated mountain-ash (*Sorbus* spp.)

None of the cankers on stone-fruit trees was typical of the diseases as they occurred on pome-fruit trees. Neither acervuli nor spores were produced and it was considered doubtful that cankers on these stone-fruit species could form naturally. The cankers found by Lawrence (*27*) were probably caused by different parasites, especially the bacterial gummosis organism *Phytomonas syringae* (Griffin) Bergey et al. (*P. cerasi* Griffin) in the case of cherry and prune.

Cankers similar to those occurring naturally on apple trees were produced by inoculations into the native Oregon crab, serviceberry, and mountain-ash. A statement by Pierce, reported by Jackson (*24*), that he had found the plant that probably is the native host of the anthracnose organism, is of interest here. The native Oregon crab might be that host plant, since it is known to range throughout the Pacific coast region in proximity to many orchards. Cankers produced on it by inoculations and those found occurring naturally are similar in all respects to those on apple trees.

Results of comparative inoculations into these three native species have been obtained. The data indicated that wild host plants have a maximum period of susceptibility similar to that in apple trees, but they appeared to exhibit little selective action toward the two fungi.

THE FUNGI IN CULTURE

EFFECT OF HYDROGEN-ION CONCENTRATION AND TEMPERATURE

Miller (*30*) reported that *Neofabraea malicorticis* and *Gloeosporium perennans* were very similar in their growth responses to temperature or to pH values of culture media. Both fungi exhibited increased growth with rise of temperature in the range of 0° to 20° C., whether inoculated into apple fruits or artificial media. He also reported that initial growth failed to occur in media at pH values of 2.0 and 12.0, while a definite retardation was apparent at 3.0 and 11.8. There was little difference between the growth of the two organisms at a given pH value, and greater variation often occurred between the different strains of a species than between the species themselves.

Touzeau [8] obtained similar results in a study of the influence of these two factors upon the growth of the two canker organisms, and his results have been substantiated in a general way by the writer. The pH values and temperature ranges offer conditions under which the various strains of the fungi may show considerable variation, but where constant distinctions between the two species fail to appear.

STANDARD MEDIA

General Considerations

More than 40 geographic or cultural strains of these organisms have been used in various experiments. They have been cultivated on a large number of media, including the standard types and many special kinds. Striking variations occurred between cultures growing on ordinary media, but these were more often intraspecific than interspecific in character. In general, when colored mycelia formed in agar cultures there was a tendency, in the majority of cases, for *Neofabraea malicorticis* to vary from white to true pinks and reds and for *Gloeosporium perennans* to vary from white to orange pinks and browns. Either organism commonly produced olive and darker pigments or mixtures of several colors. Some of these differences can be visualized from figure 4, but only a rough idea of the color variations can be presented. Characters to separate the species by simple cultural methods were not discovered.

Color Production

Cultures known to produce highly colored mycelia on certain media failed to do so when the substratum was lacking in carbohydrate, nitrogen, oxygen, or light supply. A single exposure to light for a few minutes induced the formation of colored mycelia. Compatible bacterial contaminants and some fungi usually induced formation of red pigment at the point of contact.

Conidial Formation

Typical macroconidia, as found in natural bark cankers, rarely formed in culture. When these did occur it was usually in cultures recently isolated, those transferred to radically different media, or in old tube cultures. Conidia varying in size and shape but generally smaller than true macroconidia were frequently produced in culture and are illustrated in figure 3, *A* and *B*. These were considered to be immature macroconidia, modified by cultural conditions, since in other respects they functioned as true conidia.

Microconidia are more often formed, especially from mycelia produced by macroconidia or ascospores germinated directly in water or from mycelia growing on media deficient in carbohydrates. In some cases these appeared to be budded off from macroconidia or from ascospores directly, but usually they were produced on short sterigmata, as in figure 5, *D*. This condition has been illustrated by Zeller and Childs (*42*).

[8] Touzeau, Walter D. A culture comparison of Gloeosporium perennans Zeller and Childs and Neofabraea malicorticis (Cordley) Jackson. Unpublished thesis, Univ. of Brit. Columbia. 1934.

Hyphal Coils and Cell Fusions

Several interesting structures have been observed in cultures on plain agar or in water where it was possible to follow the development of the mycelium. The young hyphae frequently became coiled at intervals, and when this occurred microconidia usually appeared on

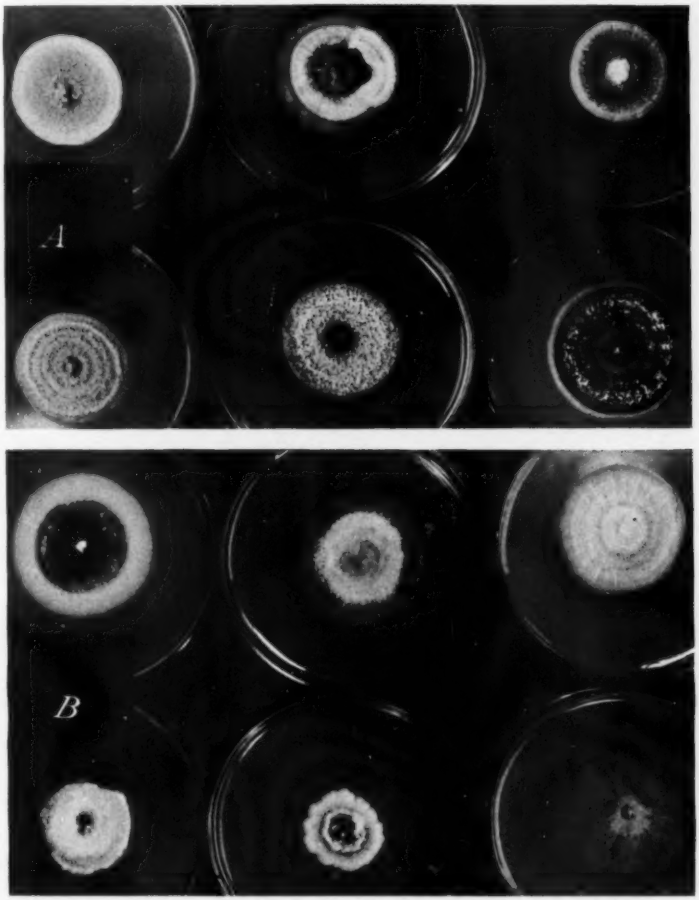

Figure 4.—Appearance of six strains of *Neofabraea malicorticis* (*A*) and six strains of *Gloeosporium perennans* (*B*), grown 20 days on Leonian's medium B at room temperature.

short outgrowths arising from the coiled structure (fig. 5, *A*). There appeared to be actual unions between certain cells of this complex, but this was difficult to determine because of the small diameter of the hyphae. In other cases it was definitely seen that cell fusions occurred, and several types of these are illustrated in figure 5, *B*. In most cases the hypha "seeking" compatible cells was very slender,

generally less than 1μ in diameter. After fusion with the receptive cell, and the frequent transfer of cell contents, this slender hypha degenerated by gradual autolysis. Cells in which fusion had taken place often formed a group of microconidia (fig. 5, *D*) or repeated the process of growth and further cell fusions. Such hyphal coils, cell fusions, and the production of microconidia in this manner are interesting from the standpoint of their suggestion of possible sexual phenomena. The microconidia bear a striking resemblance to the fertilizing cells found to be necessary for apothecial maturity in

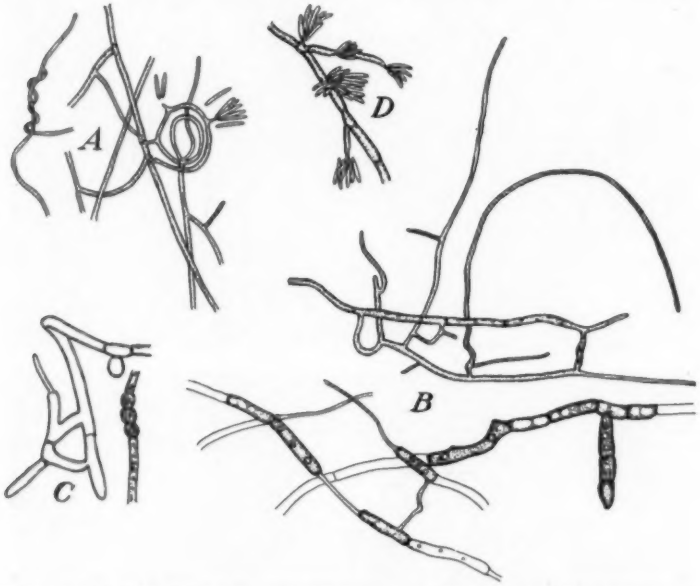

FIGURE 5.—Hyphal coils (*A*), cell fusions (*B*), resting cells (*C*), and microconidia (*D*) produced by both *Neofabraea malicorticis* and *Gloeosporium perennans* in cultures. × 312.

Sclerotinia by Drayton (*10*) and by Heuberger (*22*), and in other ascomycetes by Higgins (*23*).

RESTING HYPHAE AND OTHER ORGANS

Resting hyphae are frequently found in old cultures (fig. 5, *C*) and occasionally in natural cankers; these may serve as an additional means of overwintering or for the survival of these fungi under field conditions. Certain outgrowths commonly appeared in some cultures after they had attained maximum size. At the apexes of these structures (fig. 6) macroconidia were sometimes produced whose presence was indicated macroscopically by the mucoid character of the tips. They resembled acervuli produced in cankers from which the covering bark layer had been removed. In other cases the apexes were pressed firmly against the glass containers and appeared similar to the apothecial fundaments commonly occurring among

certain discomycetes. Since it is known that the apothecia of both the anthracnose and the perennial canker fungi originate within the stromatal mass of old acervuli, these structures may have a connection with the sexual stages in the life cycles of the organisms. The microconidia (or possible spermatia) produced in cultures have been transferred in various combinations to these possible apothecial fundaments without success in producing ascogenous organs. Since in nature these species produce their apothecia in the fall of the second season, the factors necessary for their production by artificial means may be considerably different from the requirements of the species investigated by other workers.

Conclusions

This general comparative study has failed to distinguish the fungi by common cultural methods. The macroconidia, when produced in cultures by either species under the foregoing conditions, have generally been straight and would be identified as *Gloeosporium perennans*. That typical curved conidia are produced in culture, as reported by Lawrence (*27*) and by Zeller and Childs (*42*), is not doubted, but the factors governing conidial curvature under various environmental conditions appear to be very elusive.

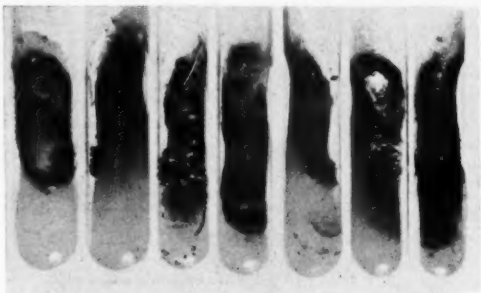

Figure 6.—Apotheciumlike structures often found in old cultures.

Special Media

Utilization of Carbon and Nitrogen From Various Sources

Eighteen isolations of *Neofabraea malicorticis* and an equal number of *Gloeosporium perennans* were selected for study of their reactions toward carbohydrate and nitrogen from various sources. All cultures were verified at least once by artificial inoculations except A–13, 176, and 177, and these were isolated from cankers typical of the respective diseases. The majority were from single-spore isolations made according to the method recently described by the writer (*26*). A few cultures from widely separated localities were included for comparative purposes. A detailed record of the cultures is given in table 1.

The use of a synthetic culture medium in liquid form for studies of this kind was found to be impracticable with these fungi. When inoculated directly into the liquid nutrient or upon buoyant materials designed to keep the fungi at the surface, the resulting growth invariably formed in part below the surface. A method of evaluating or comparing such cultures was not devised.

TABLE 1.—*Source of cultures used in a comparison of anthracnose and perennial canker fungi*

Organism and culture No.	Date isolated	Donor	Locality	Remarks
Neofabraea malicorticis:				
A-1	Sept. 29, 1931	The writer	Hood River, Oreg.	From apple rot acervulus.
A-2	1930	Nellie A. Brown	do	Canker tissue; Brown's culture No. 4.
A-4	Nov. 13, 1928	E. L. Reeves	Ferndale, Wash.	Canker tissue; Reeves' culture No. 189.
A-10	May 30, 1935	H. R. McLarty	Armstrong, B. C.	Canker tissue.
A-11a	Nov. 1, 1935	The writer	Corvallis, Oreg.	A-11a to A-11i, single conidium isolations from the same collection.
A-11d	do	do	do	
A-11h	do	do		
A-11i	do	do	do	
A-12a	Nov. 18, 1935	do	do	A-12a to A-13, single ascospore isolations.
A-12c	do	do	do	A-12c and A-12e from same apothecium.
A-12e	do	do	do	
A-12h	do	do	do	A-12h to A-12k from same apothecium.
A-12i	do	do	do	
A-12j	do	do	do	
A-12k	do	do	do	
A-12n	do	do	do	Same collection, but different apothecium.
A-12s	do	do	do	Do.
A-13	Nov. 15, 1937	do	Hood River, Oreg.	From several very large ascospores.
Gloeosporium perennans:				
P-1	Feb. 13, 1932	do	do	4 conidia from apple rot acervulus.
P-2	Sept. 29, 1931	do	do	Reisolation from apple rot acervulus.
P-3	Nov. 11, 1931	do	do	Single conidium from tree canker.
P-4	Dec. 22, 1932	E. L. Reeves	Wenatchee, Wash.	Canker tissue; Reeves' culture No. 9.
P-8	Mar. 21, 1933	R. M. Brien	New Zealand	From Delicious (?) apple rot.
167a	Feb. 15, 1935	The writer	Hood River, Oreg.	Single ascospore isolation.
167b	do	do	do	Single ascospore from same collection.
173a	Dec. 23, 1936	do	Mosier, Oreg.	173a to 173d from same collection. All single ascospore isolations.
173b	do	do	do	
173c	do	do	do	
173d	do	do	do	
174a	do	do	Hood River, Oreg.	Single ascospore isolation.
175a	Dec. 24, 1936	do	Mosier, Oreg.	175a to 175d single ascospore isolations from the same apothecium.
175b	do	do	do	
175c	do	do	do	
175d	do	do	do	
176	Nov. 15, 1937	do	Hood River, Oreg.	Multiple ascospore isolation.
177	do	do	Parkdale, Oreg.	Do.

The fungi produced hyaline, barely visible colonies on plain agar or on synthetic media solidified with agar to which no source of carbon had been added. A slight growth occurred with some strains when the medium was supplied with a source of carbon but no added nitrogen. Apparently sufficient protein material was present in the agar to supply a scant source of nitrogen for some strains of the organisms. However, when a source of nitrogen was used in such media, visible characters were outstanding in contrast to check cultures without such nitrogen. Where any doubt existed as to whether the fungi utilized a certain nitrogenous material, a nutrient medium with the desired source of nitrogen was poured upon cotton in test tubes. In this way, after inoculation a typical mycelial growth was produced which could be directly compared with the check tubes in which visible growth failed to occur.

Since sufficient protein material was present in plain agar to support some growth, a simple nutrient solution was selected to lessen the chance of introducing small amounts of nitrogen from possibly impure chemicals. The medium finally selected was a three-salt solution of half strength devised by Leonian, as reported by Groves (*17*), with the following composition: Potassium nitrate, 1 gm.; potassium phosphate (primary), 0.5 gm.; magnesium sulfate, 0.25 gm.; ferric chloride, trace; sugar or carbon source, 10 gm.; water, 1,000 cc.

To test the utilization of nitrogen, the potassium nitrate was omitted and 0.5 gm. per liter of the desired source of nitrogen was substituted, except in the case of cysteine monohydrochloride, which was added in the amount of 0.2 gm. per liter. When the medium was used in solid form 1½ percent of agar was introduced. The ability of the different strains of *Neofabraea malicorticis* and of *Gloeosporium perennans* to utilize various carbon sources is shown in table 2.

Little difference was found between the two organisms in their ability to utilize carbon from various sources, either in growth or in other features that might be useful in separating species of fungi. While striking differences between the various strains were often observed, specific distinctions were not apparent. The difference in diastatic activity between the species as reported by Zeller and Childs (*42*) was the most outstanding. When grown on starchy media, especially corn-meal or oatmeal agar, 9 strains of *Gloeosporium perennans* produced much less clearing of the medium surrounding the growth than did the 2 strains of *Neofabraea malicorticis* that were least active in this respect. Only the perennial canker culture 175b exhibited as great diastatic power as the average of 18 cultures of the anthracnose fungus.

Table 3 shows the comparative growth responses of the fungi when supplied with various sources of nitrogen. As in the previous tests, striking differences often appeared between the strains, but distinction between the two species was not possible.

TABLE 2.—*Ability of Neofabraea malicorticis and of Gloeosporium perennans to utilize carbon from various sources*

[21-day-old cultures at room temperature on Leonian's medium B]

Organism and culture No.	Growth [1] on medium B with addition of—															
	No carbon	Alcohol	Amygdalin	Arbutin	Dextrose	Dulcitol	Galactose	Glycerin	Inulin	Lactose	Levulose	Mannite	Papain	Salicin	Starch	Sucrose
Neofabraea malicorticis:																
A-1	Tr	+	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
A-2	Tr	++	++	0	++	++	+	++	Tr	++	++	++	++	Tr	++	++
A-4	Tr	+	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-10	Tr	+	++	0	++	++	++	++	Tr	++	++	++	++	+	++	++
A-11a	Tr	+	++	0	++	++	+	++	Tr	++	++	++	++	+	++	++
A-11d	Tr	+	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-11h	Tr	+	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-11i	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12a	Tr	++	++	0	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12c	Tr	++	++	0	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12e	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12h	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12i	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12j	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12k	Tr	++	++	++	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12n	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
A-12s	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
Gloeosporium perennans:																
P-1	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
P-2	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
P-3	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
P-4	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
P-8	Tr	++	++	+	++	+	+	++	Tr	++	++	++	++	++	++	++
167a	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	++	++	++
167b	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
173a	Tr	++	++	++	++	++	++	++	Tr	++	++	++	++	+	++	++
173b	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
173c	Tr	++	++	++	++	++	+	++	Tr	++	++	++	++	+	++	++
173d	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
174a	Tr	++	++	++	++	++	++	++	Tr	++	++	++	++	+	++	++
175a	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
175b	Tr	++	++	+	++	++	+	++	Tr	++	++	++	++	+	++	++
175c	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
175d	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
176	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++
177	Tr	++	++	+	++	++	++	++	Tr	++	++	++	++	+	++	++

[1] 0=no growth; Tr=trace growth; +=20 mm. or less growth in 21 days; ++=more than 20 mm. growth in 21 days. No growth of either organism on lignin or on cellulose as cotton.

TABLE 3.—*Ability of Neofabraea malicorticis and of Gloeosporium perennans to utilize nitrogen from various sources*

[21-day-old cultures at room temperature on Leonian's medium B]

Organism and culture No.	Growth [1] on medium B with addition of—																	
	No nitrogen	*dl*-Alanine	Ammonium chloride	Ammonium oxalate	Ammonium sulfate	Ammonium tartrate	Asparagine	Calcium cyanamide	Casein	Creatine	Cysteine monohydrochloride	Gelatin	*d*-Glutamic acid	Glycocoll	Peptone	Potassium nitrate	Sodium nitrate	Urea
Neofabraea malicorticis:																		
A-1	Tr	++	+	+	+	++	+	++	++	Tr	++	++	++	++	++	++	++	++
A-2	Tr	++	+	+	+	++	++	++	++	Tr	++	+	++	+	++	++	++	++
A-4	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
A-10	Tr	+	+	+	+	+	++	++	++	Tr	++	++	++	++	++	++	++	++
A-11a	Tr	++	+	+	+	+	++	++	++	Tr	++	++	++	++	++	++	++	++
A-11d	Tr	++	+	+	+	+	++	++	++	Tr	++	++	++	++	++	++	++	++
A-11h	Tr	++	+	+	+	+	++	++	++	Tr	++	++	++	++	++	++	++	++
A-11i	Tr	++	+	+	+	+	++	++	++	Tr	++	+	++	+	+	++	++	++
A-12a	Tr	++	+	+	+	+	++	++	++	Tr	++	++	++	++	++	+	++	++
A-12c	Tr	++	+	+	+	+	++	++	++	Tr	++	++	++	++	++	++	++	++
A-12e	Tr	++	+	+	+	+	++	++	++	Tr	++	++	++	++	++	++	++	++
A-12h	Tr	++	++	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
A-12i	Tr	++	+	+	+	+	++	++	++	Tr	++	++	++	++	++	++	++	++
A-12j	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
A-12k	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
A-12n	Tr	++	+	++	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
A-12s	Tr	++	+	++	+	++	++	+	++	Tr	++	++	++	++	++	++	++	+
A-13	Tr	++	+	++	+	++	++	+	++	Tr	++	++	++	++	++	++	++	++
Gloeosporium perennans:																		
P-1	Tr	++	+	++	+	++	++	+	++	Tr	++	++	++	++	++	++	++	++
P-2	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
P-3	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	+
P-4	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
P-8	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
167a	Tr	++	+	++	+	++	++	+	++	Tr	++	++	++	++	++	++	++	+
167b	Tr	++	+	++	+	++	++	+	++	Tr	++	++	++	++	++	++	++	++
173a	Tr	++	+	++	+	++	++	+	++	Tr	++	++	++	++	++	++	++	++
173b	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
173c	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
173d	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
174a	Tr	++	+	++	+	++	+	++	++	Tr	++	++	++	++	++	++	++	+
175a	Tr	+	+	+	+	+	++	+	++	Tr	++	++	++	++	++	++	++	+
175b	Tr	++	+	+	+	++	++	+	++	Tr	++	++	++	++	++	++	++	++
175c	Tr	+	+	+	+	++	+	+	++	Tr	++	++	++	++	++	++	++	++
175d	Tr	++	+	+	+	+	++	0	++	Tr	Tr	+	+	++	++	++	++	Tr
176	Tr	++	+	+	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++
177	Tr	++	+	++	+	++	++	++	++	Tr	++	++	++	++	++	++	++	++

[1] 0=no growth; Tr=trade growth; +=20 mm. or less growth in 21 days; ++=more than 20 mm. growth in 21 days.

Tolerance for Toxic Substances

The greater number of strains used in the carbohydrate- and nitrogen-utilization studies were employed to test the effects of toxic materials incorporated in media as reported by Miller (*30*) and the writer (*25*). Tannic acid was incorporated in a medium containing 4 percent of corn meal and 2 percent of agar, while the other materials were used in Leonian's medium B (*17*), to which 1 percent of dextrose and 1.5 percent of agar were added. When copper sulfate was employed, 3 percent of agar was added to obtain a solid medium. The growth reactions of the fungus strains on these media are shown in table 4.

TABLE 4.—*Growth reactions of Neofabraea malicorticis and of Gloeosporium perennans in media containing toxic materials*

[21-day-old cultures at room temperature]

Organism and culture No.	Growth[1] on media containing indicated concentration of—							
	Tannic acid		Quinone		Malachite green		Copper sulfate	
	0.5 percent	0.9 percent	0.025 percent	0.05 percent	1:400,000	1:200,000	0.05 percent	0.1 percent
Neofabraea malicorticis:								
A-1	+++	+	++	+	+	0	+	++
A-2	++	0	0	0	+	0	++	+
A-4	++	0	0	+	++	0	+	+
A-10	+++	+	0	0	+	+	++	++
A-11a	+++	+++	++	++	+	+	+++	+++
A-11d	+++	+++	0	++	+	+	+++	+++
A-11h	+++	+++	0	++	+	+	+++	+++
A-11l	+++	+++	0	0	+	+	+++	+++
A-12a	+++	+++	+++	++	+	0	++	++
A-12c	+++	+++	+++	+++	++	0	+++	+
A-12e	+++	+++	0	0	+	+	+++	++
A-12h	+++	+++	+++	+++	++	++	+++	+
A-12l	+++	+++	++	+++	++	+	+++	++
A-12j	+++	++	0	0	+	+	+++	++
A-12k	+++	++	+++	0	++	+	+++	+
A-12n	++	0	++	0	+	+	+++	++
A-12s	+++	+++	0	+++	++	+	+++	+
A-13	+++	++	++	+++	+++	++	++	+
Gloeosporium perennans:								
P-1	+++	+++	+++	+++	+++	+++	+	+
P-2	+++	+++	0	+++	++	+	+	0
P-3	+++	+++	+++	+++	+++	++	++	+
P-4	+++	+++	+++	+++	+++	+++	+	+
P-8	+++	+++	+++	+++	+	+	+	+
167a	+++	+++	+++	+++	+++	+++	++	+
167b	+++	+++	+++	++	++	++	++	+
173a	+++	+++	+++	0	++	++	++	+
173b	+++	+++	++	0	+	+	+	+
173c	+++	+++	0	++	+++	++	++	+
173d	+++	+++	+++	+++	++	+	+	+
174a	+++	+++	+++	+++	++	0	+	+
175a	+++	+++	0	0	++	++	+	0
175b	+++	+++	+++	++	++	++	++	0
175c	+++	+++	++	0	+	+	++	0
175d	+++	+++	+++	++	++	++	+	0
176	+++	+++	++	++	+	+	+	+
177	+++	+++	+++	0	+++	+++	+	+

[1] 0=no growth; +=1 to 10 mm. growth diameter in 21 days; ++=11 to 20 mm. growth diameter in 21 days; +++=more than 20 mm. growth diameter in 21 days.

Growth on media containing quinone was erratic. A depression or a stimulation of the fungi seemed to occur at the time of inoculation but was not consistent when tests were repeated.

Tannic acid inhibited the average growth rate of the strains of *Neofabraea malicorticis* to a greater degree than the strains of *Gloeosporium perennans*. The strengths used brought out no species differences, however, since several of the perennial canker cultures reacted toward tannic acid in a manner similar to the faster growing anthracnose strains.

Malachite green at dilutions of 1 to 400,000 and 1 to 200,000 inhibited the anthracnose cultures to a greater degree than the perennial canker strains. While this difference offered possibilities for separation of the species, several strains of the one were not inhibited to a greater degree than strains of the other.

Copper sulfate at concentrations of 0.05 or 0.1 percent considerably reduced the growth rates of most strains of *Gloeosporium perennans* as compared with those of *Neofabraea malicorticis*. These results are interesting, since copper sprays effectively control the anthracnose fungus in orchards but have little apparent effect upon perennial canker. The differences in mode of infection by the two organisms make it appear probable that the fungicide does not reach the infection court at the proper time in the case of the perennial canker disease.

It has been found impossible to separate the fungi by the tests reported here, but certain responses are indicated that form a basis for future study. By combining the materials showing specific inhibitive tendencies, or by further modification of some of the media, it is possible that a test may be developed suitable for taxonomic use.

VARIATIONS AND SECTORIAL MUTATIONS

General Types

The tendency of some of the cultures to produce color and growth variations was noticed early in the work. There was no difficulty in detecting sectors of the wedge- or fan-shaped type, but mutants formed also as patches, variously colored spots, or irregular sections of distinctive mycelium, which sometimes involved a considerable portion of the growth. Some of the sectors and variations observed in culture are shown in figure 7. Other characters observed may have arisen by mutation. The difficulties involved in being certain that pure lines are isolated have been discussed by Stakman et al. (*39*). Single ascospore cultures have yielded the greatest number of mutants in these tests, but this may have been accidental. The hyphal coils and cell fusions already mentioned undoubtedly offer an explanation for complex genetic characters within any one culture.

Variation of Single Strains in Apple Bark

Numerous records on canker sizes accumulated from inoculations during past years revealed confusing variations. Consistent correlations between factors such as inoculation exposures, limb diameters, tree age, and others, could not be made. General conclusions regarding susceptibility of certain types of bark tissue appeared to be warranted, but large variations often occurred in canker sizes in what appeared to be similar tree tissues.

From a knowledge of these variations, a test was designed to study the possibility of fungus variation. Young trees of the Yellow Newtown apple were selected for this purpose. On each tree 10

inoculations with the same isolate were made, starting at the base of a limb and ending near the tip. Only internodal tissue of separate trees, approximately equidistant from the lateral branches, was used, and all other factors were kept as uniform as possible. Inoculations were made December 18, 1934, and records were taken the following summer. The results of this test are given in table 5.

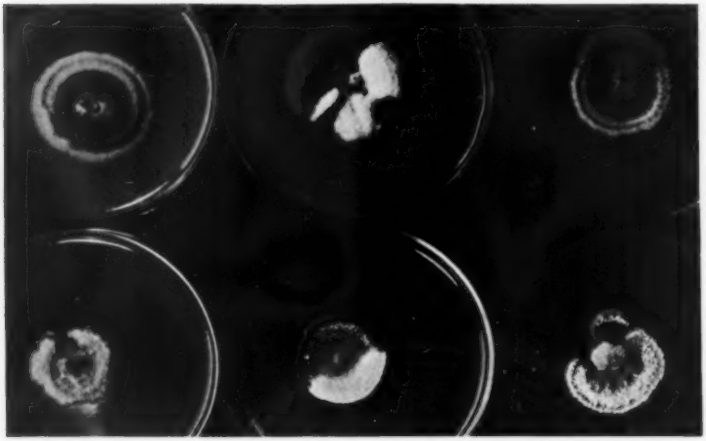

FIGURE 7.—Some types of variation produced by *Neofabraea malicorticis* and *Gloeosporium perennans* in culture.

TABLE 5.—*Variation in canker sizes from inoculations with several strains of Neofabraea malicorticis and Gloeosporium perennans into comparable apple tree tissue*

Organism and culture No.	Infection	Canker size			Organism and culture No.	Infection	Canker size		
		Minimum	Maximum	Average			Minimum	Maximum	Average
Neofabraea malicorticis:	*Percent*	*Square millimeters*	*Square millimeters*	*Square millimeters*	*Gloeosporium perennans:*	*Percent*	*Square millimeters*	*Square millimeters*	*Square millimeters*
A-1	100	[1]	800	341	P-1	100	150	720	428
A-2	30	0	150	15	P-2	100	450	600	501
A-3	100	160	900	547	P-3	100	90	700	314
A-4	100	330	1,200	763	P-4	100	[1]	300	144
A-5	30	0	270	45	P-5	100	450	800	590
A-6	0	0	0	0	P-6	100	308	1,100	734
A-7	10	0	[1]	[1]	P-7	100	375	900	695
					P-8	100	300	1,000	649

[1] Trace.

The data show that considerable variation occurred in canker sizes on what appeared to be comparable bark tissue. That the variation in sizes was not due to differences in host tissue seems reasonable, since the minimum and maximum cankers formed in irregular order. If they were due to host factors, one might reasonably expect a progressive change in sizes from the base to the tips of inoculated limbs.

Previous observations indicated that different strains might vary in their seasonal pathogenicity. A special series of inoculations with strains of known history seemed desirable to test this hypothesis

further. Young trees of the Yellow Newtown apple similar to those used in the preceding inoculation series were used, and the inoculation sites were selected as before. A series of five inoculations with each strain was made September 17, 1936, and another on November 4. Records were taken during the summer of 1937. The results are shown in table 6.

TABLE 6.—*Periodicity of pathogenicity in selected strains of perennial canker and anthracnose fungi inoculated into comparable apple tree tissue*

Organism and culture No.	Inoculated Sept. 17, 1936		Inoculated Nov. 4, 1936		Organism and culture No.	Inoculated Sept. 17, 1936		Inoculated Nov. 4, 1936	
	Infection	Average canker size	Infection	Average canker size		Infection	Average canker size	Infection	Average canker size
Neofabraea malicorticis:	*Percent*	*Square millimeters*	*Percent*	*Square millimeters*	*Gloeosporium perennans:*	*Percent*	*Square millimeters*	*Percent*	*Square millimeters*
A–1	20	26	100	538	P–1	60	156	100	714
A–2	20	34	60	47	P–5	100	607	100	1,165
A–10	80	586	100	512	167a	100	486	100	758
A–11b	60	110	100	837	167b	100	915	100	584
A–11j	0	0	100	470					

These data show that a strain weakly pathogenic at one seasonal period may become strongly pathogenic at a later period. The positive relation between canker size and low temperatures is well established for the perennial canker disease, but it can readily be seen that strains of the organism also vary in their activity at a given period. While the number of inoculations is rather small in the series tabulated, the sites were selected with special care. Numerous unpublished data further substantiate these findings.

COMPARISON OF PARENT AND SECTOR STRAINS UPON INOCULATION INTO APPLE TREES

The time required for a complete life-cycle study of these two canker producing fungi when inoculated into apple trees has limited the accumulation of data on tree-inoculation tests. Sufficiently low temperatures for suitable infections by the perennial canker organism failed to occur during the 2 years that this work was being conducted. However, data regarding the pathogenicity and conidial characters of strains derived from parent cultures have been taken. It seems desirable to present the meager data at this time, since the results have interesting implications.

The sources of the parent cultures were tabulated under a previous heading (table 1). A comparative history of the mutants and their parent cultures is given in table 7.

Each parent and mutant strain was inoculated in parallel series into young trees as the opportunity occurred. The inoculation sites were selected with special care, and all technical features were kept as uniform as possible. A record of the inoculation series is presented in table 8.

The different conidial shapes occurring in this series of inoculations, which are difficult to describe satisfactorily, are shown in figure 8.

TABLE 7.—*Characters of parent and sector strains of the anthracnose and perennial canker fungi on media*

Original species isolation	Parent or sector	Color [1] of surface mycelium [2]	Color [1] of back [2]	Source of original isolation
Anthracnose	A–2	Deep grayish olive	Dragon's-blood red	Canker tissue.
	A–2a	White	White	Spot mutant.
Do	A–12n	Greenish black and grenadine mixture.	Greenish black and Eugenia red.	Single ascospore.
	A–12na	Greenish black	Greenish black	Irregular sector.
	A–12nb	Eugenia red	Madder brown	Do.
Perennial canker	173a	Orange cinnamon, begonia rose, and glaucous zone.	Deeper shade than surface mycelia.	Single ascospore.
	173s	Cinnamon orange	Russet	Irregular sector.

[1] Colors according to Ridgway (37).
[2] 3 weeks' culture on potato 2-percent dextrose agar.

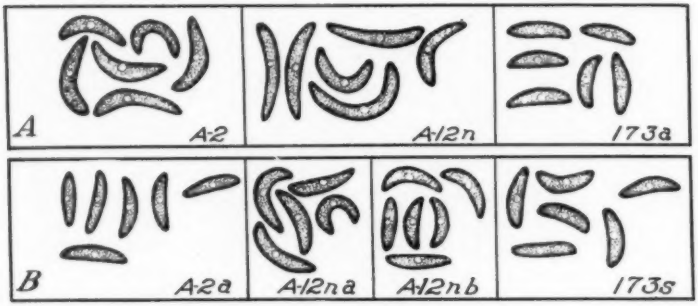

FIGURE 8—Conidia produced from inoculations into apple trees by (*A*) parent cultures, and (*B*) sector strains derived from them. ×335

TABLE 8.—*Comparison of pathogenicity and conidial characters of parent and sector strains when inoculated into young apple trees*

Culture No.	Date inoculated	Inoculations	Infection	Average canker size	Type of conidia produced on trees
	1934	*Number*	*Percent*	*Square millimeter*	
A–2	Jan. 3	14	0		
A–2a	do	14	100	282	Perennial canker.
A–2	Dec. 18	10	10	15	Anthracnose.
A–2a	do	10	100	744	Perennial canker.
	1936				
A–12n	Sept. 17	5	100	748	Anthracnose.
A–12na	do	5	80	938	Do.
A–12nb	do	5	20	40	Intermediate.
A–12n	Nov. 4	5	100	1,200	Anthracnose.
A–12na	do	5	100	1,052	Do.
A–12nb	do	5	100	1,084	Intermediate.
173a	Dec. 29	10	100	658	Perennial canker.
173s	do	10	100	259	Perennial canker (?).

While not definitely established, the results strongly suggest that the perennial canker and anthracnose diseases are caused by strains of a single fungus that may have originated by mutation. More extensive series of inoculations should be made with a greater variety of sector strains. It would be interesting to test further the pathogenicity of the straight-spored strains that have formed directly from anthracnose cultures. Fisher and Reeves (*14*) have already reported

a parasitic straight-spored strain occurring naturally. It would seem that environmental factors limit the range of the two organisms to distinct climatic districts and that one strain may arise directly from the other. Whether climatic factors cause the fungi to produce mutants that will survive only under these conditions or whether such mutants are able to extend beyond the range of the parent type is not known.

COMPARISON OF APPLE ROTS

Much of the literature on apple anthracnose and perennial canker fruit rots deals with one or the other disease or with methods for their control. The latter phase is beyond the scope of the present study.

Fisher (*11*) distinguished perennial canker rot from apple anthracnose (1) by slight color differences and by the less zoned appearance, (2) by its infection through lenticels, (3) by its appearance only after long storage, (4) by the production of more copious mycelium in a moist atmosphere, and (5) by its presence in districts where the trees were free from anthracnose cankers. Lawrence (*27*) obtained curved conidia and microspores in cultures from anthracnose apple rot tissue. In writing of perennial canker conidia, Zeller and Childs (*42*) state: "They bear a striking contrast to the characteristically curved spores of *Neofabraea malicorticis* from apple fruit." Fisher (*12*) designated the perennial canker rot as "bull's-eye rot." Several organisms were later found to cause a bull's-eye type of rot (*19*), and those caused by the perennial canker and anthracnose fungi are not distinguished at market terminals.[9] Miller (*30*) compared the temperature relations of the two organisms when inoculated into apple fruits and found them to be very similar. He stated that macroconidia were readily obtained at 10° C., but said nothing of their shapes.

In the present study many series of fruit have been inoculated with known strains of each fungus for the express purpose of observing conidial shapes. Comparative data on the rot characters and conidia on fruit have also been recorded in work on the production of spore material for tree inoculations. In addition, naturally infected fruits have been examined for several years for the purpose of determining the organism concerned.

Numerous examinations of the conidia from rotted fruit grown in the Hood River Valley have shown them to be practically straight, whether developing from known anthracnose or from perennial canker infections. These rots would be classified as perennial canker rot by present standards. Conidia of the decidedly curved type, typical of *Neofabraea malicorticis*, have rarely been encountered in these fruit-rot studies. In the majority of cases the concentric zones of color have predominated in anthracnose rots, but these and other distinguishing characters mentioned by other workers failed to distinguish the species when several strains of each were compared. One has little reason to doubt the statements of Lawrence (*27*) and of Zeller and Childs (*42*) that typical curved conidia were produced on apple fruits in their tests. The factors influencing the conidial shapes under different ecological conditions appear to be very elusive and were not discovered in the present studies. Limited trials with

[9] BRATLEY, C. O. MARKET PATHOLOGY. U. S. Bur. Plant Indus., Plant Dis. Rptr. 15: 38. 1931. [Mimeographed.]

different fruit varieties inoculated at various stages of maturity and with fruit held under different moisture and temperature conditions after inoculation yielded negative results. The moisture and temperature factors should be more fully investigated, however, since they appear to exert a major influence on the fungi in nature.

Ordinarily, sprays applied before harvest reduce the rot appearing in storage [10] (*15*, *18*, *36*). This fact suggests that infection of fruit may occur early but remain latent; or possibly the conidia do not germinate until late in the storage life of the fruit. Such infections may complicate results obtained with artificial inoculations, since mixed infections would be possible. A comparative study of the field performance of natural fruit infections by the two fungi has been impossible, for a laboratory means of certainly identifying the two remains unknown. The conidia produced by inoculating apple fruits with known cultures in some of these tests are illustrated in figure 3, *A* and *B*.

TAXONOMY OF THE FUNGI

CONIDIAL STAGES OF ANTHRACNOSE AND PERENNIAL CANKER FUNGI

The acervuli of these fungi are uniformly distributed over the surface of bark cankers. At first they are subepidermal, then erumpent, and under favorable moisture conditions expose a creamy mass of conidia (fig. 9, *A* and *D*). The stromata are composed of numerous simple or branched, septate conidiophores (fig. 9, *B* and *E*) which arise from elongated series of cells below and bear conidia at their tips. The structural features of the stromatal mass and the conidiophores appear to be identical for both organisms (fig. 9, *C* and *F*).

A review of the conidial measurements published by several workers shows little fundamental difference. Conidia of *Neofabraea malicorticis* were stated by Cordley (*7*) to measure 6μ by 24μ; by Peck (*34*), 4μ by 15μ to 17μ; and by Lawrence (*27*), 3.8μ to 5μ by 15μ to 20μ. The conidia of *Gloeosporium perennans*, according to Zeller and Childs (*42*), measure 4μ to 6μ by 12μ to 20μ. The apple anthracnose conidia have been considered to vary from curved to "hooked," while perennial canker conidia vary from straight to slightly curved. Although this has been found to be generally true, it has been pointed out previously in this paper (p. 638 and fig. 3) that intermediate forms occur which are referable to either species on the basis of these characters.

Examination of the conidia from various localities, on various hosts, in different seasons, have shown them to vary with different environmental conditions. In their typical form apple anthracnose conidia were found to be sickle- to U-shaped, comparatively sharp-pointed and tapered at the end of attachment to the conidiophores, and somewhat broadened below the apex. Typical perennial canker conidia were straight to slightly curved and evenly and abruptly tapered at each end without much variation in width. Measurements of anthracnose conidia under various conditions of development showed them to vary from 3μ to 6μ by 15μ to 35μ. In measuring for length it was possible to find sufficient conidia typical in all respects except for curvature, so that special methods, as used by Blodgett (*2*) in measuring curved spores, were unnecessary. Perennial canker conidia under similar conditions averaged shorter but ranged from 3μ

[10] See footnote 6.

to 6μ by 12μ to 25μ. Conidia of either species generally showed a spherical, hyaline spot near their centers, but might bear several guttules toward each end, be finely or coarsely granular, or contain one to several oil globules when older. The conidia often became once or twice septate upon germination.

There is, then, a condition where conidia in their typical forms are quite distinct in size, shape, and degree of curvature. In other cases, and especially in the geographical regions where the diseases overlap

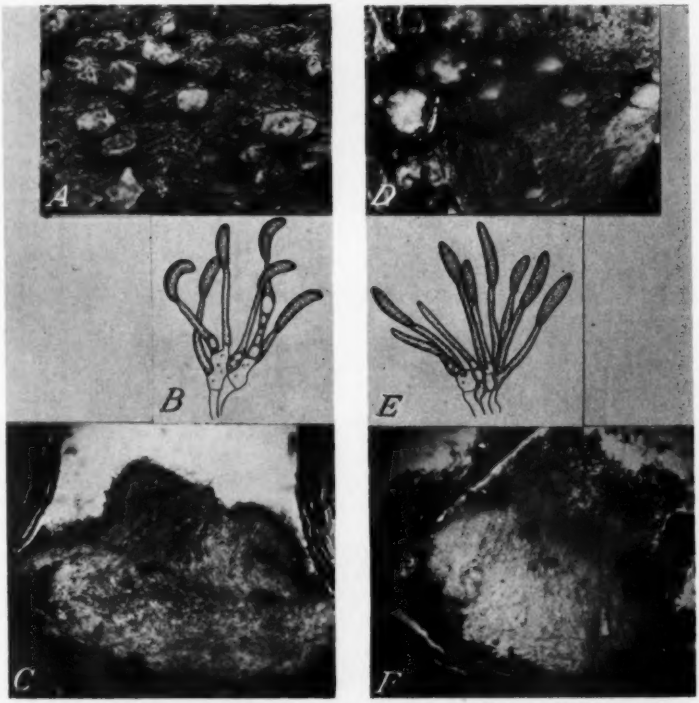

FIGURE 9.—Comparison of the perfect stages of *Neofabraea malicorticis* (*A*, *B*, *C*) and *Gloeosporium perennans* (*D*, *E*, *F*): *A* and *D*, Acervuli, × approximately 6.7; *B* and *E*, conidia and conidiophores, × approximately 355; *C* and *F*, cross section of acervuli. × approximately 67.

in range, the conidia of both species often are of intermediate form, thus making identification from the conidial characters difficult or impossible.

THE APOTHECIAL STAGE OF NEOFABRAEA MALICORTICIS

During the fall of 1935 there was opportunity to study *Neofabraea malicorticis* in its type locality, Corvallis, Oreg. Apothecia were found in abundance, at localities scattered from Corvallis to within a short distance of the Pacific Ocean. Conditions in the shore region appeared to be unfavorable for apothecial development. Moisture

seemed to be the greatest determining factor, and continued humidity after rains was as necessary as periods of rainfall. The apothecial tissues were rather fugitive and often difficult to study even when fresh.

In typical form the ascocarps were found to be from 0.5 to 1 mm. in diameter and of about the same thickness. These became exposed

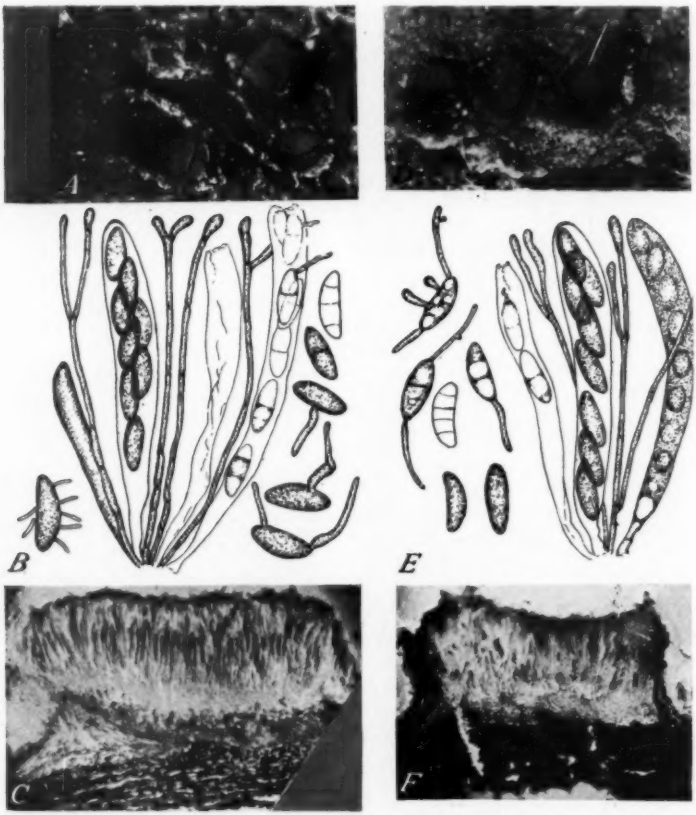

FIGURE 10.—Comparison of the perfect stages of *Neofabraea malicorticis* (*A. B. C*) and *Gloeosporium perennans* (*D, E, F*): *A* and *D*, Apothecia, × approximately 6.7; B and E, asci, ascospores, and paraphyses. × approximately 355; *C* and *F*, cross section of apothecia. × about 67.

at the surface of old conidial stromata as concave or later convex apothecia (fig. 10, *A*). Occasionally short stipes were present, especially under adverse conditions, but usually the apothecia were sessile. The blackened remains of the conidial stroma often remained clinging to the surface of the apothecium or formed a carbonaceous border around it. Asci could sometimes be found in old conidial stromata before the ascocarps pushed through the stromatic mass. The fruiting body was waxy at maturity, grayish to flesh-colored or

rosy, but gradually deliquesced by an autolytic action into a dark-colored, gelatinous, conglomerate mass. Fresh specimens darkened upon drying. The hypothecium was composed of thin-walled, loosely arranged, colorless cells, appearing like a fine net of irregular mesh (fig. 10, *C*). They merged into smaller and usually more elongated series of cells to form a thin excipular layer that was often bordered by the epidermal cells of host tissue.

Measurements of various collections from the Willamette Valley gave an average ascus measurement of 13.6μ by 102.7μ. The asci were clavate, inoperculate, sometimes slightly broader above the middle, and short-pedicellate at the base. When proper conditions prevailed, the asci elongated, discharged their spores, and then degenerated autolytically. The asci matured progressively so that a new crop of spores was ready to be expelled at each period favorable to spore discharge. Numerous hyaline, simple or branched paraphyses were interspersed among the asci (fig. 10, *B*). They were generally enlarged at the apex to as much as 6μ in diameter. The forms assumed were irregular and often bizarre. Their internal structure was finely granular.

Typical ascospores were hyaline, unicellular, ellipsoid, and often had one side somewhat flattened. The contents of the spores were either coarsely granular or contained many tiny oil globules. Germ tubes pushed out from any point in the spore wall during germination. While generally disposed in a single series, the ascospores were often partly two-seriate or somewhat crowded when about to be discharged. Measurements of 373 ascospores gave an average width of 6.4μ and a length of 19.3μ. In a degenerate state or under conditions unfavorable to spore expulsion, ascospores were commonly found to germinate within the ascus (fig. 10, *B*) and secondary conidia were often produced from them as described by Jackson (*24*). Under such unfavorable conditions, or with age, the ascospores frequently became one- to four-septate, and light amber or brownish in color. It was definitely established, however, that septate and colored spores are products of age or unfavorable environment and are not to be considered typical of the species. The entire ascus tissue became darker with age.

From this restudy of the species in the type locality and from data given in detail later in the paper, the original description is amended as follows:

Neofabraea malicorticis (Cordley) Jackson, 1913, Oreg. Bien. Crop Pest and Hort. Rpt. 1911–12: 178–197.

Synonymy: *Gloeosporium malicorticis* Cordley, 1900. Bot. Gaz. 30: 57. Also: 1900, Oreg. Agr. Expt. Sta. Bul. 60: 4–7; and 1900, Oreg. State Bd. Hort. Bien. Rpt. 6: 405–409.

Macrophoma curvispora Peck, 1900, Torrey Bot. Club Bul. 57:21.

Myxosporium curvisporum (Peck) Sacc., 1907, Wash. Agr. Expt. Sta. Bul. 83: 32–33.

Pezicula malicorticis (Jackson) Nannfeldt, 1932, Nova Acta R. Soc. Sci. Upsala., (4) 8: 91, 92.

Cryptosporiopsis malicorticis (Cordley) Nannfeldt, 1932, Nova Acta R. Soc. Sci. Upsala., (4) 8: 91.

Apothecia sessile or occasionally short-stipitate, at first concave, later flat or convex, 0.5 to 1 mm. in diameter and about the same thickness, surrounded at the edge by the blackened remains of enveloping layers, grayish to flesh-colored or rosy and waxy when fresh, becoming darker upon drying or with age, and finally gelatinizing by autolysis; hypothecium composed of thin-walled, loosely arranged, colorless, irregular cells that merge into smaller and usually more elongated series extending to form a thin excipular layer, often bordered by epidermal

cells of the host; asci clavate, inoperculate, usually slightly broader above the middle and short-pedicellate at the base, 10μ to 20μ by 75μ to 150μ (mean 13.6μ by 102.7μ); paraphyses granulose, filiform, septate, simple or branched, irregularly enlarged up to 6μ at the apex, hyaline; ascospores unicellular, ellipsoid, often somewhat flattened on one side, coarsely granular or finely guttulate, hyaline or becoming amber or brownish with age, 5μ to 9μ by 12.5μ to 26μ (mean 6.4μ by 19.3μ, mode 6μ by 20μ), often becoming one- to four-septate upon germination, one- or partly two-seriate.

Acervuli formed in cankers the first season, at first subepidermal, then erumpent, 0.2 to 1 mm. in diameter, exposing a creamy mass of conidia borne on simple or branched hyaline conidiophores; macroconidia unicellular, sometimes one-septate upon germination, sickle- to U-shaped, occasionally straight, coarsely granular to finely guttulate or with one to several oil globules at maturity, hyaline, 3μ to 6μ by 15μ to 35μ.

Developing in the fall and winter on dead bark of *Malus sylvestris* Mill., *M. rivularis* Roemer, or *Pyrus communis* L., in cankers formed by the conidial stage, *Gloeosporium malicorticis*, the previous season.

Type locality: Corvallis, Oreg.

DISCOVERY OF APOTHECIA OF GLOEOSPORIUM PERENNANS

During the course of studies with perennial canker in 1928 and 1929, J. S. Cooley, of this Division, observed an ascomycetous fungus associated with the lesions of perennial apple cankers. He studied the essential features from the meager material he was able to collect and prepared illustrations. The detailed features were very similar to those given for *Neofabraea malicorticis*, but he was inclined provisionally to give the fungus the varietal name *perennans*.[11] The description and illustrations accompanying his data clearly indicate that he had discovered the apothecial stage but that his material had developed under adverse conditions or was overmature.

A constant search by the writer was rewarded by finding apothecia resulting from artificial inoculations made on apple trees in 1932 with a known strain of *Gloeosporium perennans*. Apothecia were also discovered for the first time on pear trees in cankers resulting from artificial inoculations. These apothecia appeared in the early winter of 1935, at which time weather conditions were suitable for their formation. A further search that year yielded additional material from areas in which anthracnose cankers were absent. The following season proved to be more suitable for apothecial formation at Hood River, Oreg., and several collections of the material were made. The presence of apothecia in cankers of known origin and the collection of material in areas known to be free from anthracnose made identification relatively certain. Identification was confirmed by the frequent presence of typical conidia in the same stromata.

COMPARISON OF APOTHECIAL STAGES

A superficial examination revealed little difference between the apothecia of the two fungi (fig. 10, *A* and *D*). In their natural state apothecia of *Neofabraea malicorticis* were generally found at the center of cankered tissue or might be evenly distributed over the surface. Apothecia of the perennial canker fungus were most frequently found on the thin pellicles of bark being sloughed from the original aphis-injured and infested callus tissue common to this disease. In either case, since their forms and appearances resembled quite closely those of matured acervuli, they might easily be overlooked. A knowledge

[11] COOLEY, J. S. ASCOSPORE STAGE OF GLOEOSPORIUM PERENNANS ZELLER AND CHILDS. [Unpublished data.]

of the manner in which natural infections occur and of the appearance of cankers in their various stages is most helpful in an evaluation of specific characters.

The largest apothecial collection of *Gloeosporium perennans* was made near Mosier, Oreg., from dead apple trees that had been uprooted at least 2 years before. It was interesting to observe that ascocarps often developed in this case directly on the wood from which bark cankers had sloughed and would hardly have been suspected of being related to the perennial canker disease without the presence of adjacent intact cankers. The ascocarps under this condition were more gregarious than usual, even becoming effuse, and more irregular in shape, several occasionally coalescing to form an undulate hymenium. Apothecia of *G. perennans* developing from artificial inoculations and those forming naturally on bark tissue corresponded in size and shape to those of *Neofabraea malicorticis*. A comparison of the apothecial colors showed a tendency for translucent gray and flesh tints to predominate in those produced in perennial cankers in the dry sections east of the mountain range, while pinkish tints predominated in those from anthracnose cankers in the wetter Willamette Valley. Color differences were not consistent and formed no basis for separating the organisms. In the latter region the apothecia remained fresh longer because of moisture conditions, while in the former they were found only during rainy periods and rapidly shrank into the stromatic mass upon the cessation of the rain. Such differences in weather conditions might easily be responsible for slight variations in color.

Comparative measurements made from apothecia collected during several years are summarized in table 9, and the structural features are illustrated in figure 10.

TABLE 9.—*Measurements of apothecial material of Gloeosporium perennans and of Neofabraea malicorticis*

Organs measured and character	*Gloeosporium perennans*	*Neofabraea malicorticis*
Apothecia, diameter ..... millimeters	[1] 0.5–1	0.5–1.
Asci:		
Typical size ..... μ	14.2×102	15×110.
Range ..... μ	8.5–19.8×50–142	10–20×75–150.
Mean average ..... μ	12.64×98.1	13.6×102.7.
Ascospores:		
Number measured	465	373.
Typical size ..... μ	6.2×19.8	6×20.
Range ..... μ	4.2–8.5×11.5–22.7	5–9×12.5–26.
Mode ..... μ	5.7×17.5	6×20.
Mean ..... μ	6×17.7	6.3×19.6.
0-septate ..... percent	65.8	84.7.
1-septate ..... do	6.0	4.1.
2-septate ..... do	7.0	4.6.
3-septate ..... do	20.0	6.0.
4-septate ..... do	1.0	.4.
5-septate ..... do	.2	.2.
Paraphyses at apex, diameter ..... μ	Up to 7	Up to 6.

[1] Sometimes coalescing to form undulate hymenia.

The variation that may occur in ascospore measurements made by different workers, from different hosts, or during different seasons is shown in table 10.

TABLE 10.—*Comparison of measurements of ascospores of Neofabraea malicorticis and Gloeosporium perennans*

Organism, worker, host,[1] and locality	Ascospores measured	Mean length	Mean width
Neofabraea malicorticis:	*Number*	*μ*	*μ*
All collections by the writer	373	19.6	6.3
Willamette Valley, Oreg.	323	19.3	6.4
Hood River, Oreg.	50	21.5	5.8
Jackson's description (24)	(?)	16–19	5–7
Gloeosporium perennans:			
All collections by the writer	465	17.7	6.0
Hood River, Oreg.	290	17.6	5.7
Mosier, Oreg.	100	17.6	6.2
Parkdale, Oreg.	50	17.0	5.3
Anjou pear, Hood River, Oreg.	25	17.1	7.3
Cooley's measurements [2]	(?)	15.7	5.3

[1] Apple unless otherwise stated.
[2] See footnote 11.

An examination of tables 9 and 10 shows the close similarity between the ascogenous stages of *Neofabraea malicorticis* and *Gloeosporium perennans*. Inasmuch as the apothecia developed under different conditions of moisture and during different seasons, such variations in measurements as are shown might be expected. A separation of these forms as species based wholly on morphological or physiological grounds appears unwarranted. Because of the practical aspects of disease control, however, it seems desirable to consider them as distinct species, for the diseases caused by them must be handled as distinct diseases in the orchard. A further study of the factors influencing such characters as conidial curvature, origin of new strains, and conditions associated with their parasitic or saprophytic tendencies may eventually justify a closer combination. Cytological studies of these fungi might be very helpful in this respect.

It seems advisable at the present time to retain the specific rank of the perennial canker fungus. The specific name *perennans* is here proposed for the apothecial stage of *Gloeosporium perennans*, and the fungus is described as follows:

Neofabraea perennans (Zeller and Childs) comb. nov.

Synonymy: *Gloeosporium perennans* Zeller and Childs, 1925, Oreg. Agr. Expt. Sta. Bul. 217.

Differing from *Neofabraea malicorticis* (Cordley) Jackson in having slightly smaller asci and ascospores and in having mostly straight or slightly curved conidia. Asci 8.5μ to 19.8μ by 50μ to 142μ (mean 12.64μ by 98.1μ); ascospores 4.2μ to 8.5μ by 11.5μ to 22.7μ (mean 6μ by 17.7μ, mode 5.7μ by 17.5μ); macroconidia straight to slightly curved, occasionally strongly curved, 3μ to 6μ by 12μ to 25μ. Developing in the fall and winter on dead bark or wood of *Malus sylvestris* Mill. or *Pyrus communis* L. in cankers formed by the conidial stage, *Gloeosporium perennans*, the previous season.

Type locality: Hood River, Oreg.

A *Neofabraea malicortici* differt ascis et ascosporis paulum minoribus et conidiis plerumque rectis vel leniter curvulis. Ascis 8.5μ–19.8μ latis, 50μ–142μ longis; ascosporis 4.2μ–8.5μ latis, 11.5μ–22.7μ longis; macroconidiis rectis usque leniter curvulis, interdum valde curvatis, 3μ–6μ latis, 12μ–25μ longis. Autumno et hieme in cortice et ligno emortuo *Mali sylvestris* et *Pyri communis* in cancris a statu conidico. *Gloeosporio perennanti*, in tempore priore formatis, Hood River, Oreg.[12]

Under natural conditions the disease caused by this fungus is generally distinguished from apple anthracnose by its failure to be

[12] The writer is indebted to Edith K. Cash, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture, for the Latin description.

controlled by copper sprays and its habit of infecting injured bark tissue, especially galls produced by the woolly apple aphid and ruptured at low temperatures. Typical conidia may be distinguished by shape.

The type specimen has been deposited in the herbarium of the Department of Botany, Oregon State College, Corvallis, Oreg., and typical material in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

SUMMARY

Apple anthracnose, caused by *Neofabraea malicorticis*, and perennial canker, due to *Gloeosporium perennans* infections, are very closely related. Natural cankers are quite distinct, but the organisms could not be distinguished by means of morphological, physiological, or mycological studies.

Anthracnose occurs mainly west of the Cascade Range, and perennial canker east of that range. Both occur in certain overlapping districts, and here the causal fungi tend to be intermediate in type. Identification from the conidial characters is difficult under such conditions.

The fungi infected the same host plants when artificially inoculated. Specific separation was impossible from the characters exhibited in laboratory cultural tests on various media. Strains of the same form showed considerable difference in reaction in laboratory cultures.

Copper fungicides protect against anthracnose infections in the orchard but fail to affect perennial canker infections materially. On the other hand, the perennial canker fungus was inhibited to a greater degree than the anthracnose fungus when grown on media containing copper sulfate.

Both fungi frequently produced sectors in culture. Conidia typical of the perennial canker fungus and also intermediate forms were produced by inoculation of mutants derived from anthracnose cultures. These results suggest that the two fungi may have arisen from a single species by mutation.

A distinction between apple rots produced from inoculations with the two fungi could not be made. Conidia produced by either fungus on apple fruits were of the perennial canker type.

The apothecial stage of *Gloeosporium perennans* is described and compared with *Neofabraea malicorticis*. A slight average difference in ascospore dimensions was found, but specimens that developed under favorable environmental conditions were indistinguishable from apothecia of the anthracnose fungus. The perennial canker fungus is retained as a distinct species because of its pathological differences and is named *Neofabraea perennans*.

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FURTHER STUDIES ON ROOT CHARACTERISTICS OF WINTER WHEAT IN RELATION TO WINTER INJURY[1]

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INTRODUCTION

Previous studies on the roots of winter wheats have been reported,[2] the object of which was to find some characteristics associated with ability to survive the winter season, especially where damage from heaving was an important consideration. The experiments, although rather extensive, were of a preliminary nature. The main factors investigated were size of root, tensile strength, and stretching ability. Tests were run on the nine series given below. In all cases varieties were chosen from among those included in the Eastern Uniform Winterhardiness Nursery.

(1) The H series—preliminary studies on eight varieties.
(2) The S and 2S series—15 varieties planted in the spring, and tests run at two stages of growth (the second set incomplete). The object was to provide growing conditions widely different from the H series.
(3) The W and 2W series—fall and spring determinations on 15 varieties grown at Wooster, Ohio.
(4) The C and 2C series—fall and spring determinations on the same 15 varieties grown at Ithaca, N. Y.
(5) The pot series—grown in the greenhouse at Cornell University.
(6) The L series—a very brief study of two varieties, each grown at four fertility levels.

In a summary of the work, which extended over three seasons, the following statement was made: [3]

* * * the studies reported here indicate clearly that certain root measurements vary markedly with variety and with the resistance of the variety to cold and to heaving injury. Observations combined with the results of the experiments further indicate that no one characteristic of the roots alone is an entirely satisfactory measure of ability to resist heaving and that a number of attributes not studied as yet may have considerable significance in determining behavior.

The present paper is devoted to the further study of this problem, and reports a number of experiments supplementing those already presented.

Extensibility of roots was dropped from consideration, partly because this characteristic seemed the least important in the development of an empirical technique, and partly because difficulty was encountered in obtaining reliable data. Emphasis was put on tensile strength as affording the most promising avenue of attack. Measurement of stele diameter was made in all the new series of experiments, since it was clear that the cortex played little or not part in root strength.

[1] Received for publication February 28, 1939. Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] LAMB, C. A. TENSILE STRENGTH, EXTENSIBILITY, AND OTHER CHARACTERISTICS OF WHEAT ROOTS IN RELATION TO WINTER INJURY. Ohio Agr. Expt. Sta. Bul. 568, 44 pp., illus. 1936.

[3] See p. 43 of reference cited in footnote 2.

Five series of experiments are reported here, all from plots grown at Wooster, Ohio, in the seasons 1935–36 and 1936–37. The principal points under consideration were:

(1) Further study to find the season of the year at which samples should be taken and determinations made.
(2) A critical consideration of the value of stele measurements.
(3) The influence of level of fertility at which the crop is grown.

The technique of sampling and the details of methods were exactly as described in the earlier publication, except that data on extensibility were omitted and those on stele diameter added. With good light, the outline of the stele was discernible under the low power of the microscope, and was measured with the micrometer eyepiece. Briefly, well-developed permanent roots were selected, diameter of the whole root and vascular stele recorded, and the breaking tension determined with the special apparatus developed.

Data tabulated or calculated for each individual root were: (1) Diameter of root in millimeters; (2) diameter of stele in millimeters; (3) cross section of root in square millimeters; (4) cross section of stele in in square millimeters; (5) breaking tension in grams; (6) breaking tension in grams per millimeter of root diameter; (7) breaking tension in grams per millimeter of stele diameter; (8) breaking tension in grams per square millimeter of root cross section; (9) breaking tension in grams per square millimeter of stele cross section and (10) thickness of cortex.

Means with standard errors for each of these, and correlations between breaking tension and each of the root measurements were calculated.

PRESENTATION OF DATA

Six varieties of wheat (*Triticum aestivum L.*) were chosen for planting in the fall of 1935. Minhardi, Fulhio, and Gladden were included to provide a wide range of root size and strength, together with Thorne, Baldrock, and Purdue No. 1, three new and promising sorts developed in Ohio, Michigan, and Indiana, respectively. One series of determinations was run on these in the fall (3W series) and a second in the early spring (4W series). The results are given in table 1.

TABLE 1.—*Root, stele, and cortex measurements, and breaking-tension data from 6 varieties of wheat in fall and spring studies*

[n=200 in all cases]

Characteristic	Series [1]	Minhardi	Fulhio	Gladden	Thorne	Baldrock	Purdue No. 1
Diameter of root millimeters	3W	0.545±0.004	0.629±0.005	0.635±0.006	0.653±0.005	0.634±0.005	0.607±0.005
	4W	0.417±0.004	0.509±0.005	0.529±0.005	0.506±0.005	0.498±0.004	0.468±0.004
Diameter of stele...do	3W	0.408±0.004	0.482±0.005	0.492±0.005	0.487±0.005	0.467±0.003	0.451±0.004
Cross section of root square millimeters	3W	0.237±0.004	0.313±0.005	0.320±0.006	0.338±0.006	0.315±0.005	0.290±0.005
	4W	0.140±0.003	0.210±0.004	0.223±0.004	0.207±0.004	0.200±0.003	0.177±0.003
Cross section of stele Square millimeters	3W	0.135±0.002	0.189±0.004	0.197±0.004	0.193±0.004	0.176±0.003	0.165±0.003
Breaking tension grams	3W	240.8±4.74	396.2±8.13	438.8±6.93	394.5±7.25	460.8±7.88	323.5± 5.90
	4W	234.0±4.03	417.8±6.26	492.0±6.12	422.5±6.12	486.3±6.68	344.3± 4.53
Breaking tension:							
Per millimeter of root diameter grams	3W	439.5± 8.1	627.0±11.5	690.5±10.6	597.3± 9.4	722.5±10.5	535.0±8.91
	4W	567.5±10.2	826.8±14.1	939.5±14.0	832.8±12.7	980.0±13.7	732.8±9.30
Per millimeter of stele diameter grams	3W	593±10.4	816±14.3	891.0±14.3	803.3±12.2	979.8±14.1	715.0±11.7
Per square millimeter of root cross section grams	3W	1,040±20	1,289±25	1,422±28	1,185±20	1,474±22	1,145±21
	4W	1,768±40	2,129±47	2,294±37	2,128±42	2,535±46	2,018±35
Per square millimeter of stele cross section grams	3W	1,888±39	2,173±41	2,350±49	2,136±42	2,690±46	2,039±42
Thickness of cortex millimeters	3W	0.073±0.001	0.075±0.001	0.072±0.001	0.084±0.001	0.084±0.001	0.078±0.001
Correlations:							
Breaking tension and root diameter	3W	+0.351	+0.439	+0.283	+0.565	+0.556	+0.366
	4W	+0.112	+0.022	+0.104	+0.189	+0.259	+0.394
Breaking tension stele diameter	3W	+0.370	+0.482	+0.277	+0.570	+0.717	+0.400
Breaking tension root cross section	3W	+0.340	+0.416	+0.283	+0.567	+0.531	+0.361
	4W	+0.089	+0.039	+0.091	+0.143	+0.247	+0.378
Breaking tension and stele cross section	3W	+0.364	+0.479	+0.274	+0.552	+0.509	+0.402

[1] 3W=fall; 4W=spring.
r>0.138 significant (P<0.05); r>0.181 highly significant (P.<0.01).

At the Ohio Agricultural Experiment Station in 1928, a 3-year rotation of wheat, oats, and corn was set up at four fertility levels to levels to study the effect of soil productivity on different varieties of these crops. The results have been published elsewhere.[4] In the fall of 1935, eight varieties of wheat were grown on these plots. One replication was chosen, and samples were taken in the early winter from each variety at each fertility level for root studies. Data are presented in table 2.

Information was wanted on a number of new lines, and a special planting, which included 25 strains and varieties, was made in the fall of 1936. These were planted with the hand drill in the wheat nursery at 7-inch spacing (5W series). Ten of these varieties were repeated, and the seeds dropped by hand approximately three-fourth of an inch apart (6W series) to see whether greater uniformity of plants and a consequent reduction in variability might be obtained if seed were space-planted in the row. Data for these two series are given in table 3.

[4] STRINGFIELD, G. H., and SALTER, ROBERT M. DIFFERENTIAL RESPONSE OF CORN VARIETIES TO FERTILITY LEVELS AND TO SEASONS. Jour. Agr. Research 49: 991–1000, illus. 1934.
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TABLE 2.—*Root and stele measurements and breaking-tension data for eight varieties of winter wheat grown at four fertility levels (2L series)*

[n=100 in all cases]

Characteristic	Fertility level [1]	Minhardi	Fulhio	Gladden	Trumbull	Thorne	Test No. 1029	Dawson	Michikof
Diameter of root, millimeters	A	0.357±0.004	0.377±0.007	0.409±0.008	0.393±0.007	0.349±0.006	0.360±0.006	0.395±0.007	0.381±0.006
	B	0.374±0.005	0.432±0.007	0.486±0.006	0.496±0.008	0.390±0.006	0.459±0.007	0.459±0.006	0.406±0.006
	C	0.396±0.007	0.524±0.007	0.553±0.007	0.532±0.008	0.471±0.007	0.492±0.006	0.468±0.005	0.438±0.006
	D	0.454±0.008	0.550±0.007	0.572±0.007	0.548±0.008	0.484±0.006	0.523±0.006	0.527±0.007	0.477±0.007
Diameter of stele, do	A	0.213±0.003	0.211±0.005	0.232±0.005	0.249±0.004	0.196±0.003	0.212±0.003	0.218±0.003	0.215±0.003
	B	0.216±0.004	0.244±0.004	0.304±0.006	0.340±0.006	0.223±0.003	0.255±0.004	0.259±0.004	0.220±0.003
	C	0.218±0.004	0.327±0.006	0.323±0.006	0.355±0.007	0.271±0.004	0.285±0.004	0.267±0.004	0.242±0.004
	D	0.260±0.007	0.319±0.004	0.330±0.004	0.341±0.007	0.284±0.004	0.303±0.004	0.307±0.003	0.268±0.005
Cross section of root, square millimeters	A	0.100±0.002	0.118±0.004	0.138±0.005	0.127±0.004	0.101±0.003	0.107±0.004	0.128±0.004	0.120±0.004
	B	0.114±0.003	0.153±0.005	0.192±0.005	0.199±0.006	0.125±0.004	0.171±0.005	0.171±0.004	0.135±0.004
	C	0.131±0.005	0.220±0.005	0.246±0.007	0.229±0.007	0.181±0.006	0.194±0.004	0.178±0.004	0.155±0.004
	D	0.167±0.006	0.241±0.006	0.258±0.006	0.242±0.007	0.189±0.005	0.218±0.005	0.224±0.006	0.184±0.006
Cross section of steel, do	A	0.034±0.002	0.037±0.002	0.043±0.002	0.052±0.002	0.028±0.001	0.035±0.002	0.037±0.002	0.037±0.002
	B	0.038±0.002	0.051±0.002	0.078±0.003	0.097±0.004	0.040±0.002	0.057±0.002	0.058±0.002	0.037±0.002
	C	0.039±0.002	0.088±0.003	0.087±0.003	0.105±0.004	0.062±0.002	0.071±0.002	0.062±0.002	0.051±0.002
	D	0.058±0.003	0.087±0.002	0.090±0.002	0.097±0.004	0.068±0.002	0.077±0.002	0.077±0.002	0.060±0.002
Breaking tension, grams	A	247.0±5.95	267.0±6.00	282.5±6.85	273.5±6.40	276.5±6.25	246.0±5.40	289.5±6.05	265.5±4.99
	B	221.5±5.40	327.0±7.35	386.0±9.25	310.0±8.40	297.0±6.45	282.0±7.85	353.5±7.80	283.5±5.50
	C	221.0±5.05	450.5±10.15	429.5±8.95	339.0±10.05	318.0±7.00	348.0±8.50	341.0±7.40	306.0±5.85
	D	237.5±6.45	496.5±8.55	497.5±6.85	409.5±9.15	345.0±8.25	386.0±8.30	434.5±9.00	374.5±9.45
Breaking tension:									
Per millimeter of root diameter, do	A	691.0±16.6	722.5±18.7	698.5±17.4	706.5±19.1	795.5±22.7	704.5±18.6	750.0±19.9	708.5±16.4
	B	608.0±16.7	764.0±17.4	794.0±16.9	632.0±16.9	771.0±19.6	621.0±16.2	766.5±17.3	709.5±15.3
	C	574.0±17.1	858.0±17.8	779.5±17.2	634.0±17.1	675.5±16.8	712.0±18.6	718.5±14.5	694.5±14.6
	D	526.5±13.4	904.0±17.2	869.5±14.6	751.0±16.9	704.0±16.1	741.0±14.7	830.0±19.2	775.0±16.6
Per millimeter of stele diameter, do	A	1,133±26.1	1,339±41.7	1,219±30.6	1,098±28.8	1,414±41.6	1,179±30.1	1,349±35.1	1,249±26.2
	B	1,053±29.5	1,335±27.0	1,272±28.5	930±25.5	1,336±35.7	1,092±28.1	1,353±31.2	1,292±26.2
	C	1,033±31.4	1,397±31.3	1,338±30.1	968±27.5	1,165±27.1	1,208±30.2	1,251±22.8	1,252±25.0
	D	937±25.8	1,548±27.8	1,515±26.0	1,228±31.5	1,187±26.2	1,270±25.5	1,446±31.3	1,381±29.3
Per square millimeter of root cross section, do	A	2,541±77	2,599±105	2,266±86	2,341±89	3,030±125	2,617±103	2,532±101	2,465±92
	B	2,158±80	2,348±81	2,105±54	1,670±55	2,604±71	1,752±59	2,162±65	2,272±74
	C	1,955±87	2,118±54	1,845±55	1,549±48	1,878±61	1,841±56	1,954±46	2,056±60
	D	1,542±56	2,145±56	2,002±49	1,796±54	1,869±48	1,829±46	2,052±61	2,133±58
Per square millimeter of stele cross section, do	A	6,812±204	9,230±587	6,976±280	5,784±234	9,364±442	7,234±278	8,338±404	7,504±225
	B	6,334±255	6,980±205	5,556±199	3,598±127	7,978±316	5,430±182	6,648±197	7,600±236
	C	6,102±264	5,644±190	5,418±176	3,634±140	5,500±185	5,348±167	5,806±145	6,680±224
	D	4,976±208	6,234±152	5,924±147	4,796±179	5,252±129	5,304±129	6,052±155	6,640±207

Correlations:									
Breaking tension and root diameter	A	−0.053	+0.213	+0.355	+0.207	0.000	+0.078	+0.057	+0.090
	B	−0.101	+0.435	+0.389	+0.279	+0.138	+0.361	+0.213	+0.120
	C	+0.032	+0.354	+0.229	+0.388	+0.140	+0.170	+0.353	+0.191
	D	+0.346	+0.137	+0.113	+0.321	+0.359	+0.321	+0.043	+0.377
Breaking tension and stele diameter	A	+0.136	+0.220	+0.379	+0.236	+0.068	+0.094	+0.089	+0.193
	B	+0.020	+0.514	+0.407	+0.222	+0.075	+0.343	+0.148	+0.210
	C	−0.020	+0.284	+0.245	+0.331	+0.233	+0.129	+0.458	+0.331
	D	+0.338	+0.219	+0.148	+0.257	+0.393	+0.310	+0.133	+0.480
Breaking tension and root cross section	A	−0.043	+0.208	+0.367	+0.253	−0.011	+0.125	−0.020	+0.013
	B	−0.172	+0.409	+0.417	+0.272	+0.110	+0.382	+0.183	+0.113
	C	+0.038	+0.395	+0.211	+0.393	+0.146	+0.193	+0.368	+0.162
	D	+0.308	+0.168	+0.081	+0.349	+0.329	+0.328	+0.036	+0.388
Breaking tension and stele cross section	A	+0.059	+0.438	+0.410	+0.210	+0.142	+0.076	+0.090	+0.230
	B	+0.037	+0.477	+0.449	+0.193	+0.061	+0.274	+0.137	+0.163
	C	−0.001	+0.240	+0.247	+0.326	+0.139	+0.109	+0.427	+0.312
	D	+0.268	+0.197	+0.096	+0.264	+0.299	+0.255	+0.228	+0.564

[1] Level A received no added plant nutrient in any form and was the poorest; levels B, C, and D provided increasingly productive conditions by the addition of 1, 2, and 4 increments of fertilizer materials. For further information, see the two references by Lamb and Salter, cited in footnote 4, p. 669.

$r>0.195$ significant ($P<0.05$); $r>0.254$ highly significant ($P<0.01$).

TABLE 3.—*Root and stele measurements and breaking-tension data for 25 varieties and strains of winter wheat, 10 of which were repeated by space-planting the seeds by hand*

[$n = 100$ except where noted]

Characteristic	Series [1]	Minhardi [2]	Fulhio	Gladden [3]	Trumbull	Thorne [4]	Test No. 1029	Dawson [5]	Test No. 1048	Test No. 1055
Diameter of root.....millimeters..	5W	0.482±0.007	0.469±0.005	0.498±0.005	0.485±0.005	0.508±0.006	0.548±0.006	0.572±0.005	0.568±0.005	0.522±0.006
	6W	0.448±0.009	0.504±0.006	0.564±0.007	0.560±0.008	0.585±0.006	0.593±0.007	0.560±0.008	0.622±0.006	0.599±0.007
Diameter of stele.....do....	5W	0.371±0.006	0.348±0.005	0.390±0.004	0.355±0.006	0.384±0.005	0.420±0.005	0.433±0.005	0.439±0.004	0.397±0.006
	6W	0.291±0.007	0.379±0.005	0.434±0.006	0.410±0.007	0.455±0.006	0.464±0.006	0.419±0.006	0.468±0.006	0.449±0.006
Cross section of root, square millimeters..	5W	0.186±0.005	0.177±0.004	0.200±0.004	0.189±0.004	0.207±0.005	0.240±0.005	0.256±0.004	0.254±0.004	0.218±0.005
	6W	0.165±0.007	0.205±0.005	0.254±0.006	0.253±0.008	0.270±0.005	0.281±0.007	0.250±0.007	0.303±0.006	0.285±0.007
Cross section of stele.....do....	5W	0.113±0.003	0.099±0.003	0.123±0.003	0.104±0.003	0.118±0.003	0.142±0.003	0.153±0.003	0.155±0.003	0.129±0.004
	6W	0.072±0.004	0.118±0.003	0.153±0.004	0.137±0.004	0.168±0.004	0.173±0.005	0.142±0.004	0.177±0.005	0.165±0.005
Breaking tension.....grams..	5W	243.5±5.80	287.5±5.66	397.0±7.70	331.0±7.05	363.0±8.50	352.5±8.45	378.5±9.50	389.0±9.85	405.5±7.50
	6W	173.4±6.67	312.0±8.85	400.0±9.96	267.0±8.40	310.5±8.96	349.0±8.00	337.7±11.47	395.5±8.20	350.0±8.45
Breaking tension:										
Per millimeter of root diameter grams..	5W	503.5±13.4	611.5±12.2	788.0±15.8	684.0±14.5	709.0±16.1	642.5±16.3	651.5±15.9	687.0±17.8	779.0±14.4
	6W	381.5±15.4	612.0±17.8	706.5±18.3	474.5±14.9	530.7±16.0	588.0±13.5	600.7±19.0	638.0±14.1	582.0±14.3
Per millimeter of stele diameter grams..	5W	661±18.0	836±17.2	1,022±20.4	949±21.8	948±21.8	846±23.0	866±21.8	892±23.8	1,040±20.1
	6W	581±23.5	822±23.7	920±24.4	653±20.2	682±19.6	755±18.0	805±25.9	844±18.9	781±18.1
Per square millimeter of root cross section.....grams..	5W	1,367±49	1,672±42	2,031±49	1,813±51	1,799±48	1,518±46	1,472±37	1,562±44	1,938±48
	6W	1,102±53	1,579±53	1,638±52	1,108±40	1,191±43	1,290±35	1,386±49	1,336±34	1,274±37
Per square millimeter of stele cross section.....grams..	5W	2,344±95	3,114±90	3,380±89	3,456±116	3,236±100	2,552±94	2,574±81	2,594±81	3,416±106
	6W	2,597±131	2,856±111	2,776±100	2,090±77	1,955±75	2,090±62	2,488±94	2,346±69	2,244±64
Thickness of cortex.....millimeters..	5W	0.059±0.001	0.065±0.002	0.059±0.002	0.069±0.002	0.067±0.001	0.068±0.001	0.072±0.001	0.066±0.001	0.066±0.001
	6W	0.078±0.002	0.067±0.002	0.067±0.002	0.079±0.002	0.067±0.001	0.067±0.002	0.073±0.002	0.077±0.002	0.078±0.002
Correlations:										
Breaking tension and root diameter	5W	+0.060	+0.207	+0.141	+0.311	+0.307	+0.177	+0.337	+0.088	+0.337
	6W	+0.159	+0.054	+0.145	+0.065	+0.096	+0.302	+0.271	+0.038	+0.159
Breaking tension and stele diameter	5W	+0.163	+0.192	+0.162	+0.254	+0.335	+0.106	+0.244	+0.097	+0.312
	6W	+0.196	+0.180	+0.220	+0.121	+0.132	+0.307	+0.306	+0.049	+0.261
Breaking tension and root cross section.	5W	+0.029	+0.193	+0.097	+0.314	+0.318	+0.167	+0.319	+0.088	+0.305
	6W	+0.166	+0.045	+0.104	+0.002	+0.035	+0.246	+0.272	+0.000	+0.104
Breaking tension and stele cross section.	5W	+0.184	+0.170	+0.207	+0.302	+0.302	+0.116	+0.244	+0.045	+0.320
	6W	+0.206	+0.314	+0.171	+0.096	+0.096	+0.309	+0.346	+0.031	+0.246

Characteristic	Series [1]	Test No. 1128 [2]	Purdue No. 1	Purkof	Red Rock	Nittany	Canawa	Poole	Fulcaster
Diameter of root ..... millimeters	5W	0.536±0.007	0.517±0.005	0.468±0.005	0.569±0.007	0.602±0.007	0.520±0.005	0.555±0.006	0.588±0.005
	6W	0.521±0.006							
Diameter of stele ..... do	5W	0.398±0.006	0.389±0.004	0.364±0.005	0.440±0.006	0.464±0.006	0.397±0.005	0.424±0.004	0.454±0.005
	6W	0.377±0.005							
Cross section of root ..... square millimeters	5W	0.231±0.006	0.215±0.004	0.178±0.004	0.257±0.006	0.286±0.007	0.218±0.004	0.246±0.005	0.274±0.005
	6W	0.219±0.005							
Cross section of stele ..... do	5W	0.129±0.004	0.121±0.003	0.108±0.003	0.156±0.004	0.173±0.004	0.128±0.003	0.148±0.003	0.164±0.004
	6W	0.115±0.003							
Breaking tension ..... grams	5W	380.5±8.62	322.0±6.75	261.5±5.85	436.5±8.65	451.0±10.30	324.5±8.15	314.5±8.95	386.0±7.20
	6W	316.1±8.42							
Breaking tension:									
Per millimeter of root diameter ..... do	5W	707.5±18.1	615.5±11.3	550.5±12.6	768.5±16.2	748.0±16.4	617.5±17.0	556.0±15.7	651.5±12.5
	6W	603.5±16.6							
Per millimeter of stele diameter ..... do	5W	971±26.3	829±15.6	719±16.8	998±21.5	977±23.1	825±24.7	737±21.8	851±17.7
	6W	835±23.2							
Per square millimeter of root cross section .. do	5W	1,718±53	1,521±32	1,503±40	1,762±46	1,620±42	1,532±51	1,298±38	1,430±33
	6W	1,485±48							
Per square millimeter of stele cross section .. do	5W	3,246±122	2,750±66	2,596±80	2,970±90	2,738±83	2,740±117	2,218±78	2,432±74
	6W	2,949±112							
Thickness of cortex ..... millimeters	5W	0.073±0.002	0.068±0.002	0.058±0.001	0.067±0.001	0.070±0.002	0.066±0.001	0.068±0.002	0.071±0.001
	6W	0.077±0.001							
Correlations:									
Breaking tension and root diameter	5W	−0.058	+0.383	+0.057	+0.187	+0.288	+0.063	+0.291	+0.243
	6W	+0.140							
Breaking tension and stele diameter	5W	−0.043	+0.345	+0.152	+0.170	+0.177	−0.002	+0.169	+0.126
	6W	+0.227							
Breaking tension and root cross section	5W	−0.101	+0.333	+0.078	+0.145	+0.229	+0.072	+0.279	+0.166
	6W	+0.134							
Breaking tension and stele cross section	5W	−0.036	+0.264	+0.122	+0.179	+0.131	+0.056	+0.261	+0.116
	6W	+0.176							

$r>0.195$ significant ($P<0.05$), except where $n\pm100$; $r>0.254$ highly significant ($P<0.01$), except where $n=100$.

[1] 5W=sown with hand drill; 6W=seed space-planted by hand.

[2] $n=62$ in 6W.

[3] $n=92$ in 6W.

[4] $n=91$ in 6W.

[5] $n=73$ in 6W.

[6] $n=87$ in 6W.

TABLE 3.—*Root and stele measurements and breaking-tension data for 25 varieties and strains of winter wheat, 10 of which were repeated by space-planting the seeds by hand*—Continued

[*b*=100 except where noted]

Characteristic	Series[1]	American Banner	Test No. 1056	Test No. 1062	Hybrid selection 9	Hybrid selection 210	Hybrid selection 213	O. S. U.[2] 122-12	O. S. U.[2] 187-10
Diameter of root.....millimeters..	5W	0.571±0.006	0.554±0.004	0.587±0.008	0.612±0.005	0.599±0.005	0.601±0.006	0.514±0.006	0.563±0.005
	6W								
Diameter of stele.....do....	5W	0.443±0.006	0.420±0.004	0.441±0.007	0.471±0.005	0.460±0.004	0.464±0.004	0.385±0.006	0.434±0.004
	6W								
Cross section of root.....square millimeters..	5W	0.259±0.006	0.245±0.004	0.274±0.008	0.290±0.004	0.279±0.004	0.284±0.005	0.211±0.005	0.252±0.005
	6W								
Cross section of stele.....do....	5W	0.160±0.004	0.142±0.002	0.158±0.005	0.178±0.003	0.168±0.003	0.173±0.003	0.121±0.004	0.153±0.003
	6W								
Breaking tension.....grams..	5W	372.0±11.0	321.0±6.35	340.5±10.00	351.5±8.80	373.5±8.85	401.5±8.25	341.0±8.30	347.0±7.70
	6W								
Breaking tension:									
Per millimeter of root diameter.....do....	5W	651.0±19.2	573.5±11.3	578.0±17.2	571.5±14.1	618.0±14.1	664.5±12.7	648.5±15.9	607.0±11.7
	6W								
Per millimeter of stele diameter.....do....	5W	838±24.6	763±17.4	774±23.2	737±18.2	802±18.6	856±16.4	886±22.6	792±16.2
	6W								
Per square millimeter of root cross section...do....	5W	1,478±49	1,314±28	1,292±46	1,215±33	1,336±32	1,427±31	1,635±45	1,380±28
	6W								
Per square millimeter of stele cross section..do....	5W	2,444±84	2,320±67	2,326±101	1,994±57	2,226±57	2,354±55	3,038±106	2,324±57
	6W								
Thickness of cortex.....millimeters..	5W	0.067±0.002	0.071±0.002	0.075±0.002	0.070±0.001	0.071±0.001	0.069±0.001	0.068±0.002	0.068±0.001
	6W								
Correlations:									
Breaking tension and root diameter.....	5W	+0.155	+0.332	+0.134	+0.262	+0.341	+0.360	+0.317	+0.421
	6W								
Breaking tension and stele diameter.....	5W	+0.237	+0.192	+0.252	+0.284	+0.272	+0.317	+0.248	+0.411
	6W								
Breaking tension and root cross section.....	5W	+0.127	+0.348	+0.134	+0.296	+0.196	+0.370	+0.279	+0.459
	6W								
Breaking tension and stele cross section.....	5W	+0.219	+0.174	+0.233	+0.317	+0.270	+0.319	+0.211	+0.399
	6W								

[1] 5W=sown with hand drill: 6W=seed space-plated by hand.
[2] O. S. U. indicates Ohio State University (selection made at Columbus).

DISCUSSION OF DATA

FALL VERSUS SPRING SERIES

One of the main reasons for the 3W and 4W series was to obtain further data on the comparative values of fall and spring determinations on wheat varieties grown in the field. Mild weather with frequent showers during the fall of 1935 provided excellent growing conditions until near the end of November. In spite of somewhat late sowing (October 7), the plants entered the winter in good condition. The 3W series was run in December and the 4W series on samples from the same plots taken in March just as spring growth was starting.

In the 4W series it was not possible to measure the stele diameter with any degree of accuracy because of the confusion arising from the irregular collapse of the cortical tissue and the consequent interference with a clear demarcation of the stele outline. A comparison of the total diameter of the root in the 4W series with the stele diameter in the 3W series shows that the cortex was largely destroyed during the winter. Stele measurements on the 4W series were, therefore, discarded as unreliable.

The winter of 1935–36 was unusually severe, and both cold and heaving injury were apparent in the regular wheat nursery. The varieties included in this test, however, were not severely injured. Minhardi clearly showed the most damage from heaving. Comparison with data from earlier series is not easy, since no stele measurements had been made. However, the total root cross section in fall and in spring is available for four varieties in two seasons. The spring cross section in percentage of that found in the fall is given in table 4.

TABLE 4.—*Cross-sectional area of roots in spring in percentage of that in the fall for 4 winter wheats in 2 seasons*

Series	Season	Variety			
		Minhardi	Fulhio	Gladden	Purdue No. 1
		Percent	*Percent*	*Percent*	*Percent*
W and 2W	1934–35	61.4	84.4	83.7	75.1
3W and 4W	1935–36	59.1	67.1	69.7	61.0

With the exception of Minhardi, the cortical tissue was retained to a much greater degree in 1934–35 than in 1935–36. Minhardi, because of its smaller and weaker growth, is more liable to damage, and probably nearly all the cortex was lost in both seasons. For Fulhio and Gladden the decrease in root cross-sectional area was roughly 15 percent from fall to spring in 1934–35, and over 30 percent in 1935–36. Purdue No. 1 showed approximately 10 percent greater decreases in both seasons.

Because the cortex is subject to this considerable and variable injury during the winter, measurements of root diameter are much better made in the fall. Furthermore, when the cortical tissue collapses it makes accurate measurement of the stele almost impossible by the method used.

One advantage to be gained by using samples taken in the spring would be a possibly greater range of values for the varieties due to differential injury in the winter. There are, unfortunately, disadvantages which more than offset the gain. It would be desirable to take plants from the field after danger of further injury is past, and yet before the growth has definitely started. Apparently, however, different varieties do not all start growth at the same time, and, furthermore, conditions causing injury may occur after growth has started. The time interval that elapses while a series is run becomes a serious factor, and even if this were reduced to a minimum by storing at temperatures near freezing, the varietal differences would still introduce considerable error. An example of how serious this may be is seen by a study of data for breaking tension in the 3W and 4W series given in table 1. In table 5, the differences between the fall and spring strength of roots are given.

TABLE 5.—*Difference in breaking tension of roots of winter wheat varieties in fall and in spring*

[Data from 3W and 4W series]

Variety	Breaking tension (grams) in—		Difference [1]	Variety	Breaking tension (grams) in—		Difference [1]
	Fall	Spring			Fall	Spring	
Minhardi	240.8	234.0	−6.8± 6.22	Thorne	394.5	422.5	+28.0± 9.49
Fulhio	396.2	417.8	+21.6±10.26	Baldrock	460.8	486.3	+25.5±10.33
Gladden	438.8	492.0	+53.2± 9.25	Purdue No. 1	323.5	344.3	+20.8± 7.44

[1] Spring breaking tension minus fall breaking tension.

With the exception of Minhardi, all these differences are significant, and, with the further exception of Fulhio, approach closely or exceed the 1 percent point. Apparently, Minhardi actually suffered injury and, in addition, was probably slow in resuming growth. The large increase in strength for Gladden seems most logically explained on the assumption that this variety resumed growth earlier or grew more rapidly than the others. The remaining four varieties were very similar in their behavior.

Probably during the course of the winter, assuming no periods when active growth is resumed, there is a decrease in root strength, varying with variety and with conditions. When growth commences again, this loss is made up, and with further development the roots continue to increase in tensile strength for some time. Varieties differ in the time and rate at which growth is resumed in the spring, and this is reflected in tensile strength of the roots. Data published in the previous paper support this assumption.

Even in the fall, determinations are subject to some error when samples are taken over a period of time. The 5W and 6W data (table 3) are a case in point. These rather extensive series were started December 11; the 5W was run first and was completed on January 9. The 6W followed and was completed January 18. By December 11 growth had probably stopped, but during mild weather late in the month and early in January, certain lines, notably the named varieties

originating in Ohio and the best adapted selections, made some further growth.

Other factors, however, enter the picture, and there are differences between these two series which cannot be ascribed to the time factor alone. Variability of soil may have been responsible for some of these differences, but the plots were within 100 feet and the soil was apparently uniform. The hand-spaced seed must have been sown deeper than the other, as it was a day or two later coming up. This was unfortunate, as wet weather had delayed planting, and it was October 5 when this seed went into the ground. Before the end of the month moderately severe frosts had occurred, and the growth was probably very slow afterward. Consequently, active growth stopped just about the time the secondary roots were becoming well established, and the disadvantage of the 6W series is reflected in lower tensile strength for some varieties.

Since in neither series was the seed planted very deeply, the secondary or permanent roots arose at a point very close to the seed, and, therefore, close to the primary roots. In the 6W series it is certain that some primary roots were selected. These do not show the varietal differences evident in secondary roots, as was clearly shown in the S and 2S series. Characteristics of the primary roots are different; the stele is smaller and the cortex thicker. Their inclusion has been a serious disturbing factor, and this, combined with the fact that the permanent roots had not reached a stage of development sufficiently advanced for best differentiation between varieties, greatly reduces the value of the results. The object in including the 6W series, as previously mentioned, was to see whether spade planting might not reduce variability. Because of the disturbing factors just mentioned, the data are useless for the purpose, but are included here as illustrating the difficulties that may arise in using field-grown material for tests.

When all the data are considered, it may be concluded that the best time to take samples from the field is late fall, as soon as possible after active growth has stopped.

VASCULAR STELE MEASUREMENTS

The earlier work had shown that the cortex was almost certainly not a factor in either extensibility or strength of the root. This being so, diameter or cross section of the vascular stele should be a better measure of root size than the corresponding data on the whole root. In the 3W, 5W, 6W, and 2L series, both measurements were made, and the data have been presented in tables 1, 2, and 3. The stele diameter was somewhat more difficult to measure, but with a fairly strong light the outline was reasonably clear, provided the collapse of cortical tissue had not been too serious.

The coefficient of variability shows the stele measurements to be slightly more variable than whole-root measurements. This is due, however, to the smaller means rather than to greater range. Thickness of cortex had no correlation with stele diameter, and the range of stele diameters was nearly as great as that of root diameters. These relationships all suggest the stele as the better measure of root size to associate with breaking strength.

The tensile strength of roots varies considerably. Besides size, others factors influence the breaking tension. The earlier studies [5] showed:

> When permanent roots start, they grow very rapidly and are characterized by large diameter except very near the growing tip. Changes that take place later are concerned with lignification of the vascular tissue and endodermis. The extent to which this thickening proceeds is determined by the length of time the plant continues to grow, and during this time little or no change in diameter takes place, except possibly a reduction due to collapse of cortical tissue.

Obviously the relationship between size and strength cannot be very close under these circumstances. Still, if there is a correlation, even of small magnitude, it would be of service in indicating the value of the different size measurements. To this end, breaking tension has been recorded directly, and in addition calculated in grams per millimeter of root and stele diameter, and in grams per square millimeter of root and stele cross section. Correlations between breaking tension and each of the four root measurements were also calculated. All these data are given in the tables.

The coefficient of variability offers a satisfactory means of comparing the different methods of calculating breaking tension. In table 6 these coefficients are given for the 3W series. This series was selected because it undoubtedly provides the most reliable data. No marked improvement was obtained when the simple breaking tension was corrected to any of the root measurements. Stele diameter gave the best results, and stele cross section the worst. This is disturbing because the cross section theoretically is the more logically associated with strength. However, a study of figure 1 throws some light on the situation.

TABLE 6.—*Coefficients of variability for certain of the data on the 3W series presented in table 1*

Characteristic	Minhardi	Fulhio	Gladden	Thorne	Baldrock	Purdue No. 1	Average
Breaking tension.....gm..	27.8	22.4	22.3	26.0	24.2	26.8	24.8
Breaking tension:							
Per millimeter of root diameter....do....	25.9	25.8	21.8	22.3	20.6	23.6	23.3
Per millimeter of stele diameter....do....	24.7	24.8	22.6	21.4	20.3	23.2	22.8
Per square millimeter of root cross section.....do....	26.5	27.5	27.4	23.5	21.2	26.1	25.4
Per square millimeter of stele cross section.....do....	28.9	26.6	29.8	28.1	23.9	29.1	27.7

This graph is constructed of dots representing the actual recorded diameter, and the calculated cross-sectional area for all the classes into which the root measurements fell. Deviations of these dots from a smooth curve are due to errors introduced because of the size of the unit of measurement and more refined measurements were felt to be impracticable because of the extra time that would be required, and because error is undoubtedly introduced by the assumption that root and stele are both true cylinders. There is no object in refining measurements to the point where these errors become relatively large. From a study of the figure it is also to be noted that for the stele, using the cross section reduces the range of values considerably, and while the mean is also smaller, the reduction is not proportional.

[5] See p. 31 of reference cited in footnote 2.

It is further evident from figure 1 that within the range of values encountered for stele diameter, and even for root diameter, the area plotted against the diameter gives a curve that does not deviate appreciably from a straight line, and, therefore, that using diameter introduces but little error, even though the true relationship may be linear with area rather than diameter.

Considering the correlations, much the same argument applies. Stele diameter gave the best values and was probably superior to stele cross section. At least there is every indication that it is fully as satisfactory. The r values were lower in the latter case because there was not a reduction in standard deviation proportional to the reduction in range.

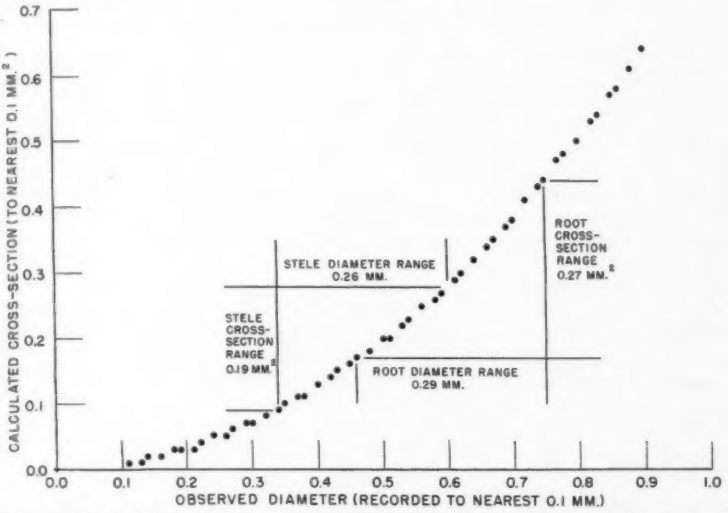

FIGURE 1.—Relation between diameter (measured to nearest 0.016 mm. and recorded to the nearest 0.01 mm.) and cross section of roots (calculated to the nearest 0.01 mm.²), and the range within which approximately 95 percent of the 3W series determinations (table 1) fell.

It has already been pointed out that growth conditions were not so good for the 5W and 6W series, and the results in general were more erratic. This was particularly true of the correlations presented in table 3. A study of scattergrams indicated that often one or a very few roots in the group of 100 would have large divergence from the mean, and would contribute a large proportion of the ($\Sigma\, dx \cdot dy$), or would reduce this total markedly. In several cases this gave abnormal r values, and the apparent inconsistencies can be discounted for this reason. The inadvertent inclusion of primary roots for some varieties was another disturbing factor.

As a whole, the data bear out the original premise that the stele is the best measure of root size to use. Stele diameter is more practical than stele cross section because of the difficulties involved in making sufficiently precise measurements to obtain accurate areas, and no serious errors are introduced by using it.

INFLUENCE OF FERTILITY LEVEL

If a test for resistance to heaving is to have any significance, it must rank varieties for a wide range of environment. No two seasons or fields are alike, and each presents its own specific set of conditions. The earlier series indicated that varieties fell in essentially the same order in several seasons. The 3W, 4W, 5W, and 6W series give further evidence that the varietal differences are consistent from 1 year to another. All these tests, however, were conducted on soil in a highly productive state. The levels of fertility rotation afforded an opportunity to study the effect of a range of soil productiveness on root characteristics. A very brief series (L series) was run on two varieties from this rotation in 1934–35, and indicated that there might be irregularities. Consequently, the 2L series was undertaken to see whether or not complications were to be anticipated from this source. Data are presented in table 2.

With but few exceptions, the results are consistent. As fertility level is raised, the roots become larger and stronger. Increased size is evident in stele as well as in whole root. However, Minhardi is distinctly out of line in breaking tension at the A (low) level. Development of the plants was very poor and secondary roots very young; in fact, in many cases they had not yet appeared. A considerable proportion of the roots used were seedling or primary roots, and this was obviously a serious disrupting factor. If this A-level figure is disregarded, the remaining data are reasonably consistent with those for the other varieties.

Even when well-adapted varieties of wheat are grown, farmers in Ohio still suffer losses from heaving. The severity of the loss depends in part on whether or not the soil lies wet in early spring, but the start that the plants have had in the fall is also very important. Since on these levels-of-fertility plots everything was sown on 1 day, the great difference that fertility level can make is clearly evident. The mean breaking tension for seven varieties (Minhardi omitted) was as follows:

	Grams
At the A level (no fertilizer)	271. 5
At the B level (1 increment of fertilizer)	319. 9
At the C level (2 increments of fertilizer)	361. 7
At the D level (4 increments of fertilizer)	420. 5

This is a wide range of values, and represents real differences in ability to withstand certain types of winter injury. It emphasizes the importance of giving wheat a good start in the fall. Yields on poorly fertilized fields are undoubtedly reduced more by winter injury than are those on more productive soils. Table 7 gives the mean yields of

TABLE 7.—*Mean grain yields of varieties of winter wheat at four fertility levels in 6 seasons*

Fertility level [1]	Average yield per acre for 11 varieties						Average for 8 varieties, 1935–36
	1928–29	1929–30	1930–31	1931–32	1932–33	Average	
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*	*Bushels*	*Bushels*
A	15.3	7.5	27.9	19.6	6.3	15.3	6.3
B	20.8	16.8	43.3	32.5	23.3	27.3	28.3
C	26.3	27.7	54.3	37.5	32.6	35.7	33.6
D	27.4	39.1	56.1	33.2	37.7	38.7	42.2

[1] See footnote, table 2.

the fertility-level plots for several seasons. It is obvious that the injury varies from 1 year to another, although in no case was any appreciable number of plants actually killed. It is not necessary, however, for plants to be killed in order that yields may be affected.

Apparently there is no reason to anticipate serious error in applying results from a test of root size and strength to a rather wide range of fertility conditions. Injury is greater at the lower levels, but the rank of varieties is not seriously changed. The series show that soil productiveness can be a very potent factor in fall development of roots. The benefits of fertilizer applications made at planting time include insurance against losses from winter injury.

SUMMARY

This paper presents data from five series of root studies, supplementing results already published. The principal points investigated were: (1) The time of year at which determinations can be made to best advantage on field-grown material, (2) the value of vascular stele measurements, and (3) the influence of fertility level on results.

Late fall was found to be the most satisfactory time to make studies. One advantage is that plants do not change so much over the period of time necessary to run the experiments. A second important point is that vascular stele measurements are much more simply and accurately made in late fall since the cortical tissue has not then collapsed seriously.

Stele diameter was found to be a better measure of root size with which to associate breaking tension than diameter or cross-sectional area of the whole root. Although cross section of stele is theoretically the best value to use, it did not give as good results as diameter. This was probably due to the fact that it is not practicable to take data with sufficient precision to give really accurate areas. No serious error is introduced by using diameter, however, because over the range in which the values of these determinations fall, the relationship between diameter and area is essentially linear.

A wide range of fertility level does not appreciably alter the rank of varieties, although it has a marked effect on both size and strength of roots.

OCCURRENCE AND LONGEVITY OF ASCOCHYTA PISI IN SEEDS OF HAIRY VETCH[1]

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INTRODUCTION

Partial to complete defoliation of plants of certain *Vicia* spp. is caused by *Ascochyta pisi* Lib.[3] Since the disease often is initiated by spores arising from seed-borne mycelium the fungus content of any seed stock materially affects its planting value. The association of *A. pisi* with seeds of hairy vetch, although not recognized by Orton (*8*),[4] has been mentioned previously by Sprague (*11*). An *Ascochyta* has been described on pods of hairy vetch (*Vicia villosa* Roth.) or of other *Vicia* spp. (*2, 12*), but specific information regarding seed infection is almost entirely wanting. According to Allescher (*1, p. 668*) and Saccardo (*10, p. 303*) a fungus designated as *Ascochyta viciae* Lib. was recognized in Europe as a caulicolous pathogen of vetch but was not mentioned as being seed borne. Another species (*A. vicicola* Sacc.) was described from both leaves and pods (*10, p. 303*) of *Vicia sepium* L., while *A. pisi* was not listed as a pathogen of any species of *Vicia*.

Sprague (*11*) isolated *Ascochyta pisi* from 9-year-old seed of *Vicia faba* L.; but while he stated that the fungus is associated with seeds of *Vicia* spp., he apparently secured no isolants from this source. Rathschlag (*9*), in studying the degree of specialization of *A. pisi* from *V. faba*, was not concerned with the occurrence of the fungus in this or other species of *Vicia*.

Wolf (*14*) definitely proved that *Protocoronospora nigricans* Atk. and Edg. infects seeds of hairy vetch but failed to report the occurrence of *Ascochyta pisi*. Apparently he confined his studies to a few selected seed stocks since *A. pisi* is common in vetch seed produced in the Southern States. McKee and Schoth (*7*) in discussing the fungus diseases of vetch omitted any reference to seed infections.

Since the presence of *Ascochyta pisi* in seeds of vetches is a little-known or recognized phenomenon and the extent and nature have been mentioned only briefly (*3*), a few data from observations and experiments of the past 5 years are presented in this paper.

EXPERIMENTAL MATERIALS AND METHODS

No attempt was made in this study to secure seed stocks of hairy vetch from severely diseased plantings. With a few exceptions all of the samples were secured from commercial offerings or from imported

[1] Received for publication March 6, 1939. Journal Paper No. 321, of the New York (Geneva) Agricultural Experiment Station.

[2] Prof. W.O. Gloyer suggested this problem and outlined the initial studies. Thanks are due to the official seed laboratories, importers, wholesalers, and retailers of forage seeds, and especially to Roland McKee, of the U. S. Department of Agriculture, for supplying the seed stocks.

[3] The binomial, *Ascochyta pisi* Lib., rather than *Ascochyta* spp. has been used throughout the paper. While the specific identity of each isolation obtained in the present study was not positively determined, the size of the pycnospores indicated that either *A. pisi* as delimited by Jones (*5*), or the imperfect stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone, or both were the only species encountered. Many isolations were grown at various temperatures on natural and artificial substrates. Since a perfect stage was never observed it was assumed that only *A. pisi* was associated with seeds of *Vicia villosa*.

[4] Italic numbers in parentheses refer to Literature Cited, p. 696.

seed stocks intended for commercial distribution. McKee drew subsamples from import lots originating in several European countries. The official seed laboratories of Arkansas, Maryland, New York, and Virginia supplied inspectors' collections and packets submitted by wholesalers. The origin of many seed-stocks could not be determined, and a number of samples represented blended bulks. In order that all sections of the United States would be represented, forage seed growers and wholesalers in several Southern and Pacific Coast States were requested to submit available stocks.

Representative pods were harvested from a few self-sown patches and commercial plantings of hairy vetch in the vicinity of Geneva, N. Y. A grower near Syracuse, N. Y., supplied tailings from his 1935 crop.

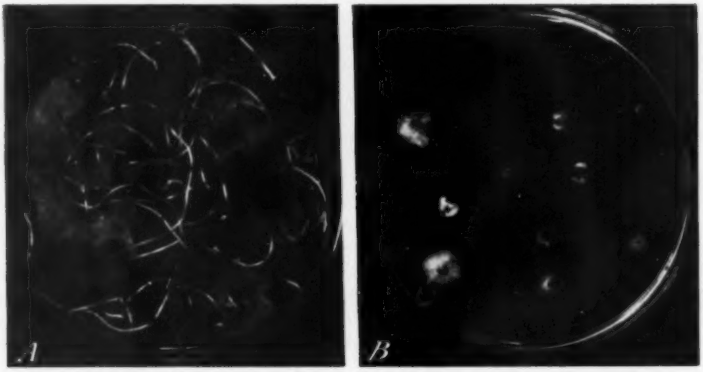

FIGURE 1.—*Ascochyta pisi* originating from surface-sterilized seeds of *Vicia villosa*: *A*, Normal seeds from a commercial seed stock; *B*, shriveled seeds from severely infected pods.

The samples were stored in a heated laboratory in their original containers. They were not fumigated or chemically treated in any manner for insect or fungus elimination. A few of the seed lots from Virginia, however, had been fumigated prior to shipment to Geneva. Portions taken from a few selected seed stocks were held at temperatures of −20°, 5°, 20°, and 35° C.

Ascochyta pisi vegetates sparsely and fruits tardily when vetch seeds are germinated on nonnutrient substrates used in seed laboratories. A mycelial growth or spore production comparable to that developing from infected peas has been observed only rarely.

Culture of surface-sterilized seeds in agar plates have made possible accurate quantitative determinations of *Ascochyta pisi*, since the fungus vegetates and fruits abundantly (figs. 1 and 2). In routine testing a mechanically counted lot of 50 seeds was surface-sterilized, placed on the agar surface with a flamed spatula, and spaced with a flamed transfer needle or by agitation.

Chloride of lime prepared according to Wilson's directions (*13*) or a commercial preparation of sodium hypochlorite was commonly used for surface sterilization. A chlorination period of 30 to 45 minutes with solutions containing 1.5 to 2.0 percent of available chlorine con-

trolled external fungi with a minimum of seed injury. A brief soak in a 0.1-percent solution of mercuric chloride eliminated surface-borne organisms but apparently inhibited growth of the internal fungi. Certain volatile mercury dusts were superior to chlorine as surface disinfectants, and their use expedited handling of the samples.

FIGURE 2.—Pycnidia and exuded pycnospores of *Ascochyta pisi* on seeds of *Vicia villosa*. × 3.

RESULTS

ASCOCHYTA PISI IN IMPORTED LOTS OF HAIRY VETCH SEEDS

In 1933 McKee submitted a group of 291 subsamples of imported vetch seed for fungus determinations. The seed stocks were grown in, or exported from, several European countries during 1929, 1930, 1931, and 1932. At least 900 seeds from each sample were grown on agar plates during 1934. Only 1 of the 181 seed stocks imported from 10 countries during 1929 and 1930 was shown to contain *Ascochyta pisi*. As shown in table 1, 3 countries were the principal exporters to the United States in 1931 and 1932. The presence of *A. pisi* was demonstrated in seed from only these countries, and from a fourth country from which seed was imported in 1932 only.

Seed of the 1933 crop from Hungary received at Geneva in March 1934 produced fungus colonies in October 1934 while seed from Lithuania did not.

TABLE 1.—*Ascochyta pisi in seeds of Vicia villosa imported from several European countries*

Origin of shipment	Entries of 1931			Entries of 1932		
	Samples received	Samples infected		Samples received	Samples infected	
		January 1934	October 1934		January 1934	October 1934
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Czechoslovakia	1	0	0	2	0	0
Germany	19	0	0	8	2	0
Hungary	32	2	0	4	0	0
Latvia	14	4	0	10	7	5
Lithuania	0	0	0	3	1	0
Sweden	3	0	0	2	0	0
All others	12	0	0	0	0	0

The results obtained from growing surface-sterilized seeds received from official laboratories, wholesalers, and other agencies are summarized in table 2. The origin of many seed stocks was unknown. Several were mixtures of imported lots, and others were compounded from imported and domestic lots. This is especially applicable to the New York samples, as it was definitely known that many of them were imported, and in 1936 at least 18 were labeled as being representative of European-grown winter vetch. All but one of the New York samples produced growths of *Ascochyta pisi*, and the percentage of infected seeds varied from 1 to 4. Several lots of domestic origin contained 2 to 4 percent, and one lot at least 9 percent, of infected seeds.

A grower in central New York annually submitted a marketable sample for germination and disease determinations. *Ascochyta pisi* was isolated each year. The appearance of the seed did not suggest internal infections. Heavy rainfall in late spring apparently increased the percentage of fungus-seed associations, especially when the rye support crop failed to prevent matting of the vines.

TABLE 2.—*Presence and longevity of Ascochyta pisi in seeds of Vicia villosa stored in a heated laboratory since receipt at Geneva, N. Y.*

State where seed was grown or sold	Year when harvested	Samples received	Samples containing *A. pisi* in tests in—							
			March 1934	November 1934	September 1935	July 1936	April 1937	November 1937	April 1938	November 1938
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Arkansas	1935	6			1	1		1		0
Illinois	1935	1			0	0		0	0	
	1936	2				1	1		0	0
Maryland	1934	22		18		13	2	1		
Michigan	1932	2	1	0		1	0	0		
	1933	8	5	4	3	2			1	
	1935	2			2	2	2			1
	1936	2				2	2		2	
New York	1931	5	2	2		1	1	1	1	
	1932	18	13	12		8			5	
	1933	17		10	10	6	4		3	2
	1934	8			8	8			7	7
	1935	29				24		24		15
	1936	15					11		11	12
	1937	2							2	2
Oregon	1935	4			1	1	0	0		
	1936	1					0	0		
South Carolina	1933	2	2	2	1	2			0	0
Virginia [1]	1932	4	2	3		2	0	0		
Do. [2]	1932	11	8	6		3		2		
Do. [3]	1932	18	10	8		4		0	0	
Do. [4]	1933	3	1	0		0				
Do. [5]	1933	22	13	5		0		0		
Do. [6]	1933	4	4	1		1	1	0	0	
Wisconsin	1933	3		3		1	0	0		
Importers [7]	1934	13			5	4		2		0

[1] Seed of European origin.
[2] Seed grown in Virginia or adjacent States, not fumigated.
[3] Seed grown in Virginia or adjacent States, fumigated.
[4] Samples obtained from importations from Hungary.
[5] Samples obtained from importations from several European countries.
[6] Seed received in Virginia in spring of 1934, origin unknown.
[7] Samples obtained from 13 importers of European seed.

The data in table 2 show that several States either produce or plant infected seed. That *Ascochyta pisi* is particularly common to the seed in any State or States cannot be proved. In a few samples from

Maryland, believed to represent imported seed stocks, the percentage of fungus-seed associations exceeded 10. Infections in 15 percent of the seeds were noted in 1 imported and in 1 locally grown sample collected in Virginia. Since the optimum temperature for vegetation, spore production, and germination is above 25° C., it appears logical that the disease would be more severe in the Southern than in the Northern States. The fungus, however, develops at lower temperatures, and the percentages of infected vetch seedlings in fields in New York have always increased during winter or early spring. Undoubtedly moisture is more often a limiting factor in pod infections than is temperature.

LONGEVITY OF ASCOCHYTA PISI

The absence of demonstrable fungus growths from European vetch imported in 1929 and 1930, as mentioned previously, is not conclusive evidence that *Ascochyta pisi* was not present originally. Presumably phenological conditions could vary sufficiently from year to year to cause differences in the pod and, therefore, seed infections. The assumption that fungus-seed associations were established but that the fungus later died out is more tenable. The data in table 1 are inferential evidence of this assumption as is also the fact that when the 1932 entries from Latvia were examined in 1937 *A. pisi* could not be demonstrated in a single seed.

The samples received in the period from 1933 to 1938 were tested soon after their receipt and again on the dates noted in table 2. In general there was a decrease in the number of infected samples at each succeeding examination. Apparently the fungus retains its viability in a few seeds for a period of at least 7 years but usually dies out of seed stocks held in heated storage within 4 or 5 years.

That the temperature of storage affects the longevity of *Ascochyta pisi* was indicated in one experiment. Portions of a well-cleaned seed stock grown in New York in 1934 and of an imported lot presumably harvested in 1934 were stored in Sealright containers at −20°, 0°, 20°, and 35° C. during January 1935. Both seed stocks were air-dried to a moisture content of about 9 percent. The former contained 3 percent and the latter 4 percent of infected seeds in tests in December 1934. When surface-sterilized and grown on agar in March 1938 the percentages of fungus-seed associations at the various temperatures for the New York sample were: −20°, 1.2; 0°, 2.5; 20°, 1.5; and 35°, 0.5. For the imported lot at the same temperatures, they were: −20°, 2.5; 0°, 3.5; 20°, 0.2; and 35°, 0.5.

The percentage of viable seeds in the samples did not decrease materially at the three colder temperatures, whereas at 35° C. the germinations were originally 95.5 and 93.5 percent, and in 1938 46.0 and 30.0 percent for the New York and imported samples, respectively.

In experiments with New York samples a fumigant, carbon bisulfide, apparently did not reduce the percentage of seeds producing growths of *Ascochyta pisi*. Fumigation of small quantities of vetch collected in Virginia, however, may have eliminated the fungus from a few of them. As shown in table 2 about 56 percent of the fumigated and 73 percent of the untreated samples developed colonies of *A. pisi* when tested about 20 months after harvest. The original percentages

of infected samples could not be determined. Since the germination of every sample approximated 90 percent in March 1934, the fumigant could not have materially affected the viability of the seed.

COMPARISON OF LONGEVITY OF FUNGUS WITH LONGEVITY OF SEED

The decline in percentage of culturable fungus growths was not paralleled by a corresponding depression in germination of the seed. As is evident in table 2 *Ascochyta pisi* inside the seed may die within only 2 or 3 years and rarely remains alive longer than 6 or 7 years. Furthermore, the number of growths obtained from 200 test seeds of any sample usually decreased at each successive date of testing. For example, samples Nos. 3319, 4010, and 5908 from Virginia contained 15, 15, and 6 percent, respectively, of infected seeds in March 1934; 8, 6, and 4 percent in November 1934; 0, 2, and 1.5 percent in November 1936; and 0, 0, and 0 in July 1937. The germination percentages on artificial substrates in March 1934 were No. 3319, 94; No. 4010, 96; and No. 5908, 95. In July 1937 the percentages were 88, 91, and 92, respectively.

An imported sample germinated 94 percent in 1932 and 88 percent in 1937, whereas the fungus content decreased from 15 percent in 1932 to 0 in 1936.

Many other seed stocks retained their vitality for a number of years even though *Ascochyta pisi* died out entirely. On the other hand the germination of a few samples dropped rapidly while the percentage of infected seeds remained more or less constant for several years.

McKee and Schoth (7) stated that properly dried seed suffers little or no decrease in germination in 5 years and often no decrease in a longer period. In tests at Geneva 9 lots from Virginia contained from 86 to 90 percent of viable seed after 4 years in heated storage. Another group of 21 samples stored for 5 years germinated from 9 to 98 percent, with an average of 71 percent.

RELATION OF IMPERMEABILITY OF SEED COATS TO FUNGUS-SEED ASSOCIATIONS

That hard seeds materially affect the percentage of fungus growths developing on agar plates has not been demonstrated. The seeds that remained impermeable to water in 14-day tests of several lots were clipped with an electric needle and tested for *Ascochyta pisi*. The fungus developed in several plates but more often did not, and developed only rarely from a higher percentage of seeds than the results from unselected seeds indicated.

Studies with a large bulk drawn from a local crop harvested in 1934 showed that the incidence of *Ascochyta pisi* was greater originally in the promptly germinating than in the hard seeds. In this particular seed stock the hardness of the seed did not increase the longevity of any internal fungus.

Hard seeds to some extent decreased the accuracy and duplicability of the data in table 2. In counting the number of fungus colonies and computing the percentage of infections, the number of impermeable seed was not usually considered. Since *Ascochyta pisi* rarely emerges until swelling has occurred, it is possible that clipping the hard seeds would have increased the apparent fungus-seed associations. However, when a considerable number of seeds remained impermeable during the 20-day test period, the percentage of infections was based on swollen seeds alone.

RELATION OF POD SPOTS TO INFECTED SEEDS

Obviously, the seeds harvested from pods free of pod spot should also be disease-free. However, the development of *Ascochyta pisi* from normal seeds void of any external sign or symptom of disease suggests the possibility of systemic infection. In order to prove the relation of pod spots to fungus-seed associations, collections of pods from two garden patches were separated into diseased and clean groups and the seeds examined for the presence of *A. pisi*.

The pods from 1 patch were collected before maturity, and consequently the seeds were so abnormal as to appear diseased. In a total of 103 seeds from 28 pods only 1 maintained its spherical shape upon drying and only this 1 germinated. As expected, none of the pods free of external spots contained infected seeds. However, *Ascochyta pisi* originated from only 4 of the 73 seeds harvested from diseased pods. The 4 seeds were adjacent to spots covered with pycnidia. The use of a surface disinfectant apparently did not prevent the development of *A. pisi* since 2 infected seeds had been surface-sterilized, the other 2 had not.

Either hyphal masses or proliferated tissue from the inner walls of diseased portions of pods were removed and placed on agar surfaces. Scrapings from 20 pods seemed not to contain *Ascochyta pisi* although many colonies of *Penicillium* spp. and *Rhizopus nigricans* Ehr. developed.

In a larger collection from 14 plants it was found that 519 pods were spotted while 2,278 were not. After a period of 15 months the pods were again separated into groups based on the presence of definite lesions with numerous pycnidia, with very small lesions, and with no lesions. The seeds were carefully removed and classified as shown in table 3. They were mechanically counted in lots of 25, surface-sterilized for 25 minutes in a solution containing 1.8 percent of available chlorine, and spaced on surfaces of potato-dextrose agar.

The results (table 3) indicate that seeds are infected through the pods and that disease-free seed stocks can be obtained by pod selections. To assume that the presence of pod spots is proof that the seeds are infected is erroneous. Only 5.1 percent of the seeds of commercial size from pods with lesions were shown to contain *Ascochyta pisi*. Many of the pods were covered with spots and pycnidia, as many as 25 distinct spots being noted on several. If only 1 infected seed were obtained from a pod, the 5.1 percent shown in table 3 would have accounted for about 26.0 percent of the total number of spotted pods.

TABLE 3.—*Germination and fungus content of vetch seeds obtained from pods spotted or not spotted with Ascochyta pisi*

Condition of pods	Seeds cultured	Germination	Seeds giving rise to—	
			A. pisi	Contaminants
	Number	*Percent*	*Percent*	*Percent*
Small lesions, no pycnidia	225	50.6	0.88	1.33
Definite lesions, spherical seeds	500	76.0	5.10	1.80
Definite lession, shriveled seeds	100	5.0	4.00	2.00
No lesions, hand-threshed seeds	500	71.0	0	1.20
No lesions, self-threshed seeds	200	81.5	0	.50

Presumably the seeds that escaped from self-threshed pods were more nearly mature than those threshed by hand since a higher percentage of the former produced normal sprouts. That fewer contaminants were obtained from this group might also be an indication of greater maturity.

LOCATION OF ASCOCHYTA PISI IN SEEDS

Wolf (*14*) demonstrated by a study of sectioned vetch seeds that hyphae of *Protocoronospora nigricans* may permeate all parts of the seed but did not mention the location, or even the presence, of *Ascochyta pisi*. When macerated portions of shriveled seeds were examined in the present study, hyphae of either *A. pisi* or *Alternaria* sp. or both were found in all parts of a few seeds. When *A. pisi* occurred in normally shaped seeds, it apparently was limited to the seed coats and the cotyledons. The plumules and radicles were not shown to be infected, and the fungus did not develop from them in agar platings. Furthermore, *A. pisi* rarely vegetates around ungerminated seeds and then only when they have swollen and the seed coats have become ruptured or deteriorated.

A number of seeds were soaked in water, and as soon as the seed coats had softened they were removed from the cotyledons. After a short period of sterilization in a chlorine solution both seed coats and cotyledons were placed on agar surfaces. Only 1 colony of *Ascochyta pisi* developed from the cotyledons of 250 shriveled seeds while 3 originated from the seed coats. Platings of entire seeds showed about 8 percent of infection.

Studies with other lots also proved that fewer fungus growths developed from surface-sterilized seed coats and cotyledons than from entire seeds. *Ascochyta pisi* vegetated around 1 decorticated seed and 1 seed coat in a sublot of 150 seeds of which 3 percent were previously shown to be infected. From another group of 50 seeds harvested from spotted pods 3 fungus colonies developed around the cotyledons while none originated from the seed coats. *A. pisi* vegetated around 5 percent of the unmutilated seeds. Surface sterilization apparently killed *A. pisi* in both the cotyledons and seed coats of several other test samples.

ELIMINATION OF ASCOCHYTA PISI BY HEAT TREATMENTS

Attempts to kill *Ascochyta pisi* either while inside the seeds or after it has emerged have not been highly successful. The results from heat treatments of dry or moist seeds were inconclusive. Immersion in water at 60° C. for 10 minutes reduced the percentage of seed infections without depressing germination, while a 13-minute soak killed the seeds but neither *A. pisi* nor *Alternaria* sp. was eliminated. Increasing the exposure period to 16 minutes effected complete kills of both seed and fungi.

When held in a drying oven at 70° C. for 9 days the germination of one lot of imported vetch was reduced from 92 to 85 percent, and after 16 days to 0. The presence of *Ascochyta pisi* was detected in seeds only to the seventh day. A domestic lot similarly treated was killed in 11 days, but pure cultures of *A. pisi* were secured from seeds treated for 12 days.

Steamed wheat grains harboring *Ascochyta pisi* were included in this experiment. In one group the percentage of grains producing growths of the fungus after 0, 1, 7, 11, 15, and 17 days were 80, 81, 37, 21, 29, and 0, respectively. The fungus was eliminated in 11 days from steamed grains with an originally higher moisture content. Pure cultures, however, were obtained from 23 percent of the grains exposed for 9 days.

Small samples from blended bulks collected locally were killed in 1 day at 90° C. No seed was demonstrated to contain living mycelium. Wheat grains, however, were sterilized only after an exposure of 5 days. Fragments of infected pods were also included in these trials. The pycnospores which exuded promptly were placed to germinate at 25°. The percentages of germination for spores representative of the 0-, 2-, 5-, 7-, and 10-day periods were 98.5, 74.3, 37.5, 0.06, and 0.0, respectively.

Neither seeds, seed-borne mycelium, nor mycelium in dried wheat grains survived storage at 120° C. for 1 hour. An air temperature of 145° completely killed two samples of winter vetch in 30 minutes. Colonies of *Ascochyta pisi* did not arise from the surface-sterilized seeds. The viability of a well-dried domestic seed stock was depressed by exposure at 145°. The germination was 96 percent for the controls, 73 percent for the seeds exposed 30 minutes, and 0 for the seed heat-treated for 60 minutes. *A. pisi* also was destroyed in 60 minutes, but vigorous colonies originated from 2 percent of the seeds subjected to the 30-minute treatment.

CHEMICAL SEED TREATMENTS FOR CONTROL OF ASCOCHYTA PISI

The influence of certain chemical protectants on the germination of, and fungi in, vetch seed was studied with four infected lots. Similar portions of each seed-stock were treated with the materials listed in table 4 in December 1935. They were immediately planted in greenhouse beds either in soil previously cropped with wheat, in electrically heated soil, or in a mixture of washed sand and virgin muck, and also in the laboratory on wood-pulp blotting paper.

As shown in table 4, no chemical materially reduced the percentage of germinating seeds or emerged seedlings. Zinc oxide was definitely beneficial in regard to germination and to emergence and vigor of the seedlings.

Leaf infections were observed in a few rows, and might have developed in others had not the seedlings been discarded only 20 days after emergence. The appearance of *Ascochyta pisi* in the blotter tests also indicated that chemical dusts will not entirely prevent fructification of the fungus on the surface of seeds.

Shriveled seeds removed from a domestic bulk were treated with the chemicals listed in table 4. *Ascochyta pisi* developed on at least 5 percent of the seeds on every blotter irrespective of the kind or dosage of chemical. New Ceresan eliminated mold fungi and reduced, but did not eliminate, the percentage of *A. pisi*-bearing seeds. The other dusts were noticeably less efficient in mold control and effected no significant reduction in *A. pisi*-seed associations in this particular lot. The seeds were stored in sealed bottles; and when they were germinated again in October 1938, *A. pisi* was detected only on the seeds treated with 1.0-percent cuprous oxide and those treated with 1.5-percent zinc oxide.

TABLE 4.—*Relation of kind and dosage of fungicide to germination of seeds of hairy vetch placed on various substrates*

[Data are averages from four seed stocks]

Chemical and dose (percent)	Emergence from seeds planted in—			Germination on blotters	
	Common soil	Heated soil	Virgin muck	December 1935	October 1938
New Ceresan:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0.054 [1]	78	84	77	95	96
0.081	76	78	76	94	96
0.108	[2] 77	80	84	97	98
0.172	74	[3] 78	80	95	95
Mercuric chloride:					
0.027	78	[3] 82	79	94	96
0.054	84	85	76	98	98
0.108	82	85	[2] 74	[4] 96	96
Copper carbonate: [5]					
0.27	74	83	83	[4] 95	93
0.68	77	84	81	97	[4] 96
Cuprous oxide:					
1.00	77	80	77	[4] 95	96
1.50	[3] 73	87	77	[4] 98	97
Zinc oxide:					
1.00	[3] 82	87	88	98	97
1.50	[3] 85	87	87	97	96
Controls	76	83	78	96	[4] 96

[1] Equivalent to a dosage of one-half ounce of dust per bushel of seed.
[2] Seedlings infected with *Ascochyta pisi*.
[3] Several seedlings damped-off.
[4] *A. pisi* detected on seeds.
[5] Copper content stated to be 50 percent.

The influence of the various dusts on the development of *Ascochyta pisi* from four infected lots is shown in table 5. The seeds were treated in December 1935 and promptly stored in screw-top jars at room temperature. They were germinated on agar plates in June 1936. No material prevented the development of the fungus. New Ceresan, however, controlled the mold fungi, while the other chemicals were less efficient.

The data in table 5 further indicate that New Ceresan effected a significant decrease in the percentage of *Ascochyta pisi*-seed associations of the lots stored for 3 years. Presumably this reduction in fungus growths is the result of the action of gaseous ethyl mercuric phosphate during storage. The untreated seed was also stored in sealed bottles and was tested in October 1938. New Ceresan as a 0.2-percent dip did not affect the development of *A. pisi*.

At the beginning of this study chlorine soaks and ethyl mercury phosphate both as an instant dip and as a dust were compared with untreated seed to determine their effect on the presence of *Ascochyta pisi*. At least 200 seeds of each of 24 seed stocks were treated either by soaking for 90 minutes in 0.5-percent chlorine solution, by dipping in a 0.3-percent New Ceresan suspension in water, or by dusting with New Semesan Jr. *A. pisi* developed from 16 of the soaked, 10 of the dusted, 5 of the dipped, and 9 of the untreated tests. The average percentages of infected seeds were 2.6, 1.4, 0.7, and 1.0, respectively.

REDUCTION OF FOLIAGE INFECTIONS BY SEED TREATMENTS

Proof that leaf spot of vetch is initiated by seed-borne mycelium as well as evidence that it is difficult to control was obtained from a planting of 6 infected seed stocks in 1936 and 1937. Samples representative of both foreign and domestic seed stocks were selected for

their known fungus content and mechanically divided into 8 subsamples. They were treated either by soaking in hot water for the exposure periods listed in table 6, by dusting with Ceresan at the rate of 2½ ounces per bushel of seed, or by treating with a dust composed of 1 part New Ceresan and 4 parts cupric stearate by weight. The approximate adherence rate of this mixture was 4 ounces per bushel of seed. The treated lots were weighed to provide approximately 500 seeds for each row and were planted on August 8 in shallow trenches spaced sufficiently far apart to prevent dissemination of spores. Adjacent growths of hairy vetch originating from disease-free seed were not infected by *Ascochyta pisi*.

TABLE 5.—*Influence of kind and dosage of chemical on development of Ascochyta pisi and of saprophytic fungi in four seed stocks of hairy vetch treated in December 1935*

Chemical and dosage [1] (percent)	Infected seeds, June 1936		Infected seed stocks, October 1938		Infected seeds, October 1938	
	A. pisi	Saprophytes	*A. pisi*	Saprophytes	*A. pisi*	Saprophytes
New Ceresan:						
0.054	3.0	1.0	1	1	0.5	0.5
0.081	3.5	1.0	1	1	1.0	.5
0.108	3.0	.5	0	1	.0	.3
0.162	3.0	.5	1	1	.4	.3
Mercuric chloride:						
0.027	4.0	9.5	3	4	4.0	10.8
0.054	7.0	10.0	4	4	3.7	7.5
0.108	6.0	8.5	3	4	1.5	6.8
Copper carbonate:						
0.270	2.5	6.7	3	4	4.8	6.7
0.680	6.0	2.2	3	4	3.5	10.7
Cuprous oxide:						
1.00	3.0	12.2	2	4	3.0	2.7
1.50	3.0	14.7	3	3	5.7	3.0
Zinc oxide:						
1.50	3.5	12.8	3	4	3.5	20.3
2.00	3.0	10.8	3	3	3.8	9.7
Stored untreated: [2]						
Check	5.5	21.5	2	4	4.2	24.5
New Ceresan dip [3]			4	1	5.8	.2
Sodium hypochlorite [4]	5.0	1.8	4	2	4.8	.5

[1] Dosage is based on ratio of dust to seed expressed in percent by weight.
[2] Seed was stored untreated and then treated as indicated immediately before placing on agar plates.
[3] A 0.2-percent solution containing 0.01 percent of the active ingredient, ethyl mercuric phosphate.
[4] A solution testing 1.8 percent of available chlorine.

TABLE 6.—*Relation of hot-water soaks and of certain chemicals to the emergence, vigor, and leaf-spotting of plants of hairy vetch*

Seed treatment	Temperature	Soaking period	Index of growth [1]	Length of stems	Plants infected	
					October	April
	°C.	*Minutes*		*Inches*	*Percent*	*Percent*
Untreated			100	12	1.8	9.5
Hot water	50	50	90	11	.1	2.1
	50	75	85	11	0	1.9
	55	45	70	10	.1	1.4
	55	60	65	9	0	1.7
	60	12	115	13	.6	8.3
Ceresan			100	10	0	5.7
New Ceresan [2]			5	8	0	.8

[1] Comparative mass of vegetative growth in April 1937.
[2] Mixture of New Ceresan and cupric stearate.

Scattered leaf spots were noted in most of the rows of untreated seed during September, and in increasingly greater numbers each month until the plants were removed. All of the rows were examined critically during October and April. After the latter examination the plants were pulled and stored so that the progression of stem infections could be observed. The data summarized in table 6 are based on records of both field and dried-plant examinations for the six seed stocks. While they are only comparable, the data very clearly illustrate the influence of the various treatments upon the growth of, and disease present on, vetch seedlings.

OTHER FUNGI IN SEEDS OF HAIRY VETCH

While only *Ascochyta pisi* is commonly obtained from hairy vetch, other fungi are occasionally present and were encountered in this investigation. An unidentified species of *Alternaria* developed from surface-sterilized seeds in nearly 9 percent of the samples. In a portion of a domestic seed stock received in 1937, 52 colonies of this fungus originated from plantings of 400 seeds while only 12 colonies of *A. pisi* were recorded. Usually fewer seeds in any particular sample were infected with *Alternaria* sp. than with *A. pisi*. The former fungus was more tolerant of mercury compounds than the latter and often vegetated profusely even when *A. pisi* appeared to be injured.

Fusarium spp. were of infrequent occurrence in commercial seed stocks, being found only nine times. A planting at Syracuse, N. Y., produced heavily infected seed. Apparently moisture is very essential in the establishment of this seed-fungus association. It is, of course, probable that spores or mycelium were carried externally, as has been reported for peas (*4*, *5*, *6*), and were destroyed by surface sterilization.

Rhizoctonia solani Kühn was isolated from a single sample. The fungus was shown to be pathogenic to beans and peas as well as to several species of vetch. McKee and Schoth (*7*) reported that *Rhizoctonia* sp. seriously damaged plantings in Florida.

A mildly pathogenic fungus tentatively assigned to the genus *Xylaria* was secured from an imported seed-stock received in 1935. It completely destroyed seedlings growing on agar, while *Ascochyta pisi* under similar conditions merely discolored portions of the stems and infrequently invaded the tips.

FUNGI IN SEEDS OF OTHER VICIA SPECIES

Samples of a number of other vetches have been examined from time to time. *Ascochyta pisi* was not demonstrated in seeds of *Vicia atropurpurea* Desf., *V. dasycarpa* Ten., *V. ervilia* (L.) Willd., *V. monantha* Desf., *V. ludoviciana* Nutt., and *V. pannonica* Grantz. supplied by McKee. A single sample of *Vicia monantha* from South Carolina, and three samples of *V. atropurpurea* and two of *V. pannonica* from Oregon were proved to be infected.

Plants of *Vicia sativa* L. are susceptible to *Ascochyta pisi*, but the fungus was not isolated from any of 21 seed stocks. A typically clean sample dipped in a suspension of New Ceresan and then placed on an agar plate is shown in figure 3. *Alternaria* sp. was isolated from two samples and *Fusarium* sp. from one sample of *V. sativa* (fig. 4).

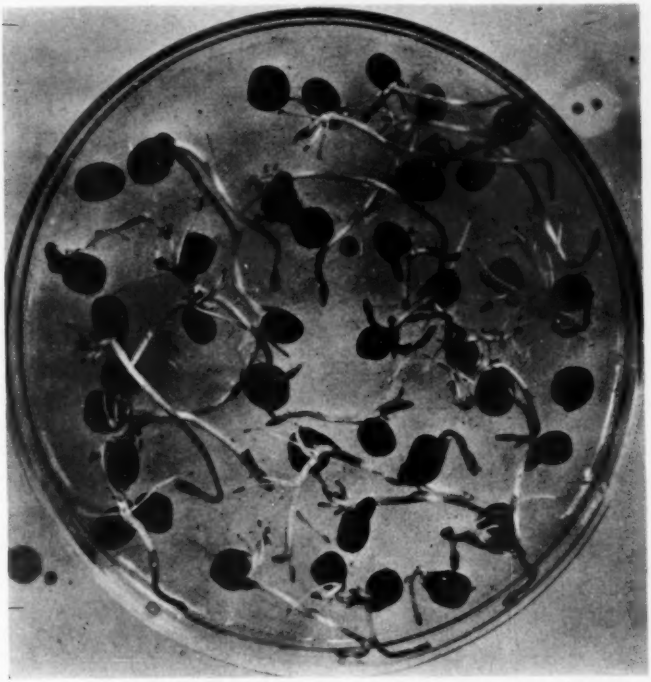

FIGURE 3.—Seeds of *Vicia sativa* surface-sterilized with New Ceresan. × 1.

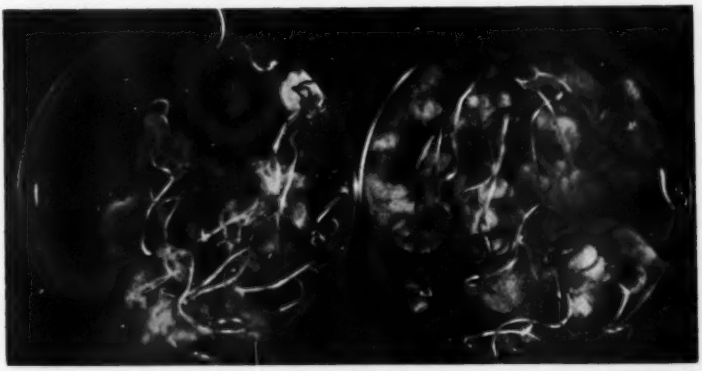

FIGURE 4.—*Alternaria* sp. originating from surface-sterilized seeds of *Vicia sativa*. × 2/3.

SUMMARY

In 1934, 181 lots of hairy vetch seeds imported in 1929 and 1930, and 110 lots imported in 1931 and 1932 were surface-sterilized and cultured on agar plates. *Ascochyta pisi* was demonstrated in only 1 of the former; 16 of the latter seed-stocks showed infection in January 1934.

A total of 224 samples representative of both domestic and foreign crops of 1931 to 1937, inclusive, were secured from various agencies in 9 States. When tested immediately after their receipt, 149 of the samples were infected internally. During storage in a warm, dry laboratory for 1 to 5 years the number of *Ascochyta pisi*-seed associations decreased continuously.

The percentage of viable seeds remained nearly constant for 4 years or more, thus suggesting prolonged storage as a natural process of fungus elimination.

Longevity of the fungus could not be correlated with a hard-shell condition of the seed. A definite relationship existed between infection in pods and seeds.

The organism is usually resident immediately inside the seed coats but may occur in all parts of severely infected seeds.

Both spores and mycelium of *Ascochyta pisi* were less affected by exposures to hot air or water than were vetch seeds.

The fungus content of seed-stocks treated with New Ceresan decreased significantly during a 2-year storage period. Other chemicals were ineffective in eliminating internal fungi.

Alternaria sp., *Fusarium* spp., *Rhizoctonia solani* and *Xylaria* sp. were isolated infrequently from seeds of *Vicia villosa*. *Ascochyta pisi* may be resident in seeds of several *Vicia* species but not in those of *V. sativa*.

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FURTHER STUDIES OF THE EFFECTS OF TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS UPON THE PHOTOPERIODIC RESPONSES OF PLANTS [1]

By R. H. ROBERTS, *professor of horticulture, Wisconsin Agricultural Experiment Station*, and B. ESTHER STRUCKMEYER, *research assistant in horticulture, University of Wisconsin*

INTRODUCTION

The marked effect exerted by temperature on the photoperiodic response of many of the higher plants has already been reported.[2] During the winter of 1937–38 an intermediate or "medium" temperature house with a minimum night temperature of 63° F. was maintained in addition to the "warm" house kept at 70° and the "cool" house kept at 55° which had been used the previous year. Most of the observations were made between October and April, when the day temperatures and the night temperatures remained relatively uniform. From the results obtained during the past several seasons it appears, as Hamner and Bonner [3] reported for *Xanthium*, that night temperatures are more effective than the accompanying day temperatures in determining the nature of the response that many kinds of plants will make. So far, a control of only the night temperatures during the winter months has proved to be consistently effective in modifying the influence of photoperiod on plants.

EXPERIMENTAL PROCEDURE

In 1938–39 the minimum temperatures employed were 55°, 65°, and 75° F. This rise in the warmer-temperature houses was used to accentuate the effect of warm temperature in restricting the flowering of such short-day plants as tobacco and poinsettia.

Long- and short-day conditions were provided in each of the three temperature houses. The long-day environment was secured by the use of 100-watt lamps from just before sunset until midnight. These were so placed as to deliver from 30 to 80 foot-candles of light to the plants. Prior to November 1 and after March 1, short days of 9½ to 10 hours were maintained. No artificial shortening of the day was employed during midwinter.

Six or more plants of a variety were used in each treatment. The different lots received similar cultural and moisture treatments. When variability in growth within a lot was apparent, the plants that were photographed represent the average condition. In the lots that were relatively uniform representative samples were selected at random. As will later be shown, the amount of variability is largely a matter of the growth response of each species to a particular environment.

[1] Received for publication February 13, 1939. The work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] ROBERTS, R. H., and STRUCKMEYER, B. ESTHER. THE EFFECTS OF TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS UPON THE PHOTOPERIODIC RESPONSES OF SOME OF THE HIGHER PLANTS. Jour. Agr. Res. 56: 633–677, illus. 1938.

[3] HAMNER, KARL C., and BONNER, JAMES. PHOTOPERIODISM IN RELATION TO HORMONES AS FACTORS IN FLORAL INITIATION AND DEVELOPMENT. Bot. Gaz. 100: 388–431, illus. 1938.

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Except as indicated, the data recorded for the plants in table 1 were obtained from 2 to 10 repeated plantings. The results from repeating a treatment have been very consistent. The reason for this is that care was taken to have plants from a similar source. It is to be shown later, for example, that plants from cuttings and from seedlings give unlike responses to photoperiod at certain temperatures.

It is believed that the length of time required for a plant to react to a photoperiodic treatment should be given relatively little weight in attempting to determine the response of a plant to photoperiod. The

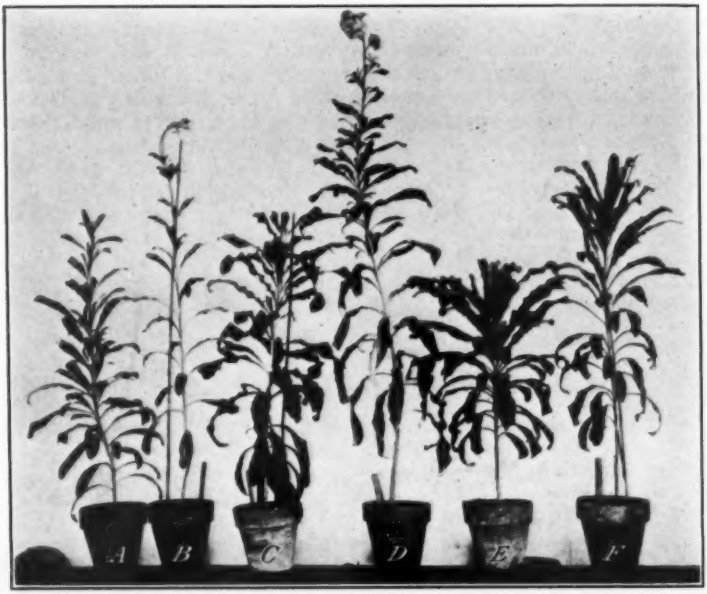

FIGURE 1.—Stock, which is a long-day plant at an intermediate temperature (*D*): *A*, Cool, short-day (budding); *B*, cool, long-day (blossoming); *C* intermediate temperature, short-day (nonblossoming); *D*, intermediate temperature, long-day (blossoming); *E*, warm, short-day (nonblossoming); *F*, warm, long-day (nonblossoming).

time required for a plant to flower, though sometimes very important commercially, is of little interest in cataloging the photoperiodic reactions of plants. The important item technically is not whether a plant comes to flower quickly but, rather, whether it soon acquires the growth characteristics that are readily recognized as being associated with blossom production. One lot of plants may come to flower much more quickly in one location than another lot in a different location, although blossom primordia may have been initiated at practically the same time in each lot. It seems confusing to speak of the first group as being more responsive to photoperiod than the second group. When classifying plants as to their photoperiodic type, the later as well as any immediately apparent reaction should have its proper emphasis.

EFFECT OF TEMPERATURE ON THE RESPONSE OF PLANTS TO PHOTOPERIOD

The addition of a third temperature environment produced a number of results which were not apparent in the earlier experiments where only two temperature levels were maintained. One example is that of stock, variety Xmas pink (fig. 1). This plant was a long-day plant

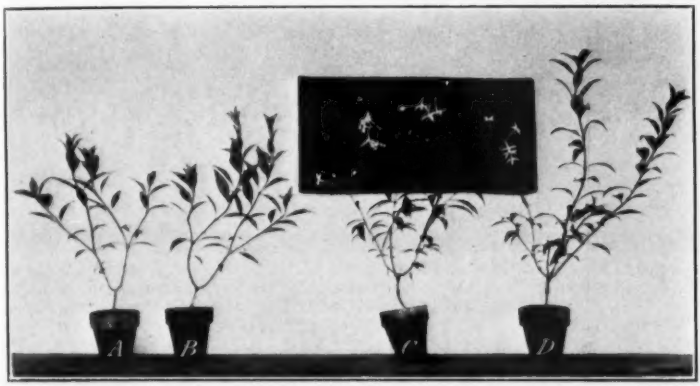

FIGURE 2.—*Bouvardia humboldti*, which blossoms quickest in warm, short days; plants in long days blossom at some growing points in longer periods of time; *A*, intermediate temperature, short day; *B*, intermediate temperature, long day; *C*, warm, short day; *D*, warm, long day.

when grown at an intermediate temperature. In a cool location the plants in short days as well as in long days blossomed, although those in short days were slower in coming to flower. Neither the lot in short days nor that in long days flowered in the warm location. Post [4]

FIGURE 3.—Christmas cactus, which needs an intermediate temperature as well as a short day to induce early blossoming (*C*): *A*, Cool, short day; *B*, cool, long day; *C*, intermediate temperature, short day; *D*, intermediate temperature, long day; *E*, warm, short day; *F*, warm, long day. Plants in cool, long days sometimes form buds.

has previously called attention to the effect of temperature upon the reaction of stock to photoperiod.

The response of nasturtium, variety Golden Gleam, was similar to that of stock. Morning-glory, variety Heavenly Blue, blossomed in

[4] POST, KENNETH. SOME EFFECTS OF TEMPERATURE AND LIGHT UPON THE FLOWER BUD FORMATION AND LEAF CHARACTER OF STOCKS (MATHIOLA INCANA). Amer. Soc. Hort. Sci. Proc. 32: (v. 33): 649-652. 1935.

FIGURE 4.—Maryland Mammoth tobacco, which fails to fruit in short days if the temperature is too warm: *A*, Outdoors, short day (budding); *B*, outdoors, long day; *C*, greenhouse, short day (remaining vegetative); *D*, greenhouse, long day. Grown from seed produced by plants in long days at cool temperature. Photographed August 19.

short days at a warm-temperature, in both long and short days at an intermediate temperature, and in long days (after a long time) at a cool temperature. Similar effects of temperature in modifying the response to photoperiod were noted for a number of species, as table 1 and figures 2 and 3 show.

Maryland Mammoth tobacco is generally regarded as a strictly short-day plant. Apparently this view is the result of the plants hav-

FIGURE 5.—*Nemesia versicolor*, which blossoms in a wide range of environments: *A*, Cool, short day; *B*, cool, long day; *C*, intermediate temperature, short day; *D*, intermediate temperature, long day; *E*, warm, short day; *F*, warm, long day. The plants at a warm temperature, especially in long days, show a chlorotic condition (*F*).

ing been grown under a limited range of temperature conditions, as this variety becomes fruitful in long days as well as short when grown at a minimum temperature of 55° F. Plants given short days at a very warm temperature remain vegetative and fail to blossom (fig. 4). Thus this variety not only fruits in long cool days, but fails to fruit in short days at a high temperature. Seed produced in cool long days grew into plants that gave typical short-day reactions at an intermediate temperature.

Nemesia versicolor (fig. 5) and annual larkspur (*Delphinium ajacis*) responded only slightly to photoperiod. The date of flowering was delayed at the cool temperature, but the time at which the type of

TABLE 1.—*Influence of temperature upon the photoperiodicity of some of the higher plants*

Plant	Nonfruitful (Nf) or flowers (F) produced at temperature of—						Remarks
	70° or 75° F. (warm)		63° or 65° F. (intermediate)		55° F. (cool)		
	Short day	Long day	Short day	Long day	Short day	Long day	
Alfalfa (*Medicago sativa*)	Nf	Nf [1]	Nf	F	F [3]	F [3]	3 clons.
Barley (*Secale cereale*)	Nf	Nf [1]	Nf	F	F [3]	F [3]	2 varieties.
Beet (*Beta vulgaris*)	Nf	Nf [1]	Nf	Nf [4]	F [3,4]	F [3]	3 varieties.
Begonia semperflorens	Nf	F	F	F	F [3]	F [3]	
Bluegrass (*Poa pratensis*)	Nf [1]	Nf	Nf	Nf	F [3]	F [3,4]	
Bouvardia humboldti	F [3]	F [3]	Nf	F	Nf [1]	Nf	See fig. 2.
Chinese cabbage (*Brassica pekinensis*)	Nf	Nf [1]	Nf	F [4]	F [3]	F [3]	
Christmas cactus (*Zygocactus truncatus*)	Nf [1]	Nf	F [3]	Nf	F [4]	F [3,4]	See fig. 3.
Chrysanthemum morifolium var. Lillian Doty.	F [3]	Nf	Nf	Nf	Nf [1]	Nf	
Chrysanthemum morifolium var. Golden Sceptre.	F [3]	Nf	F	Nf	Nf [1]	Nf	
Cocklebur (*Xanthium echinatum*)	F [3]	Nf	F	Nf	Nf [1]	Nf	10 weeks.
Corn (*Zea mays*)	F [3]	F [3]	F [4,5]	F	(6)	(6)	
Cornflower (*Centaurea cyanus*)	Nf	F	Nf	F	F [3,4]	F [3]	
Cosmos sulphureus var. Klondike	Nf [1]	Nf	F [3]	Nf	F [4]	Nf	
Dianthus barbatus	Nf	Nf [1]	F [3]	F [3]	F [4]	F	
Dogfennel (*Anthemis cotula*)	Nf	F [3]	Nf [4]	F	F [3]	F	
Gourd (*Luffa cylindrica*)	Nf	F [3]	F [3]	F	(6)	F [4]	
Jimpsonweed (*Datura stramonium*)	F [3]	F [3]			Nf [1]	Nf	
Kohlrabi (*Brassica oleracea caulo-rapa*)	Nf	Nf [1]	Nf	F [4]	F [3]	F [3]	
Mallow (*Malva verticillata*)	F [3]	Nf	F	F [3]	Nf [1]	Nf	
Marigold (*Tagetes erecta*)	(6)	F	(6)	F [3]	F [3]	F	
Morning-glory (*Ipomoea purpurea*) var. Heavenly Blue.	F [3]	Nf	F	F [3]	Nf [1]	F [3]	
Naegelia cinnabarina	F [3]	F [3]	F [4]	Nf	(6)	(6)	1 lot.
Nasturtium (*Tropaeolum majus*)	Nf	Nf [1]	Nf	F [3]	F [3]	F	
Peas (*Pisum sativum*) var. Canada field	Nf	Nf [1]	Nf	F [3]	F [4]	F	
Peas (*Pisum sativum*) var. Wisconsin Early.	(6)	Nf	Nf	F [4]	F [3]	F [3]	
Pennycress (*Thlaspi arvense*)	Nf	Nf [1]	Nf	Nf	F [3]	F [3]	
Petunia hybrida	Nf	F	Nf	F [3]	F [3]	F	
Poinsettia (*Euphorbia pulcherrima*)	Nf [1]	Nf	F [3]	Nf	Nf [1]	F [3,4]	
Primrose (*Oenothera* sp.)	Nf	Nf [1]	Nf	F [4]	Nf	F [3]	
Red clover (*Trifolium pratense*)	Nf	Nf [1]	Nf	F [4]	Nf	F [3]	
Rudbeckia laciniata	Nf	F [3]	Nf	F	F [3]	F	
Salvia splendens var. Harbinger	F [3]	Nf	F	Nf	F	F [3]	
Snapdragon (*Antirrhinum majus*)	Nf	F	Nf	F [3]	F [3]	F	
Soybeans (*Soja max*) var. *Bilasi*	F [3]	Nf	F	Nf	Nf [1]	Nf	
Soybean (*Soja max*) var. "Chippewa"	F [3]	F [3]	F [4]	F	Nf	Nf [1]	
Spinach (*Spinacia oleracea*)	Nf	Nf [1]	Nf	F	Nf	F [3]	
Stock (*Mathiola bicornis*)	Nf	Nf [1]	Nf [4]	F	F [3]	F [3]	See fig. 1.
Timothy (*Phleum pratense*)	Nf	Nf [1]	Nf	F [4]	Nf	F [3]	
Tobacco (*Nicotiana tabacum*) var. Maryland Mammoth.	Nf [1]	Nf	F [3]	Nf	F	F [3]	See fig. 4.
Vetch (*Vicia sativa*)	(6)	(6)	Nf	F	F [3,4]	F [3]	
White clover (*Trifolium repens*)	Nf	Nf [1]	Nf	F [4]	Nf	F [3]	3 varieties.
White clover (*Trifolium repens*) var. Louisiana White.	Nf	F	F [4]	F [3]	F [3]	F	Cuttings.

[1] Flowering suppressed by temperature.
[2] Flowering induced by temperature.
[3] Typical photoperiod reaction.
[4] Some tendency to flower.
[5] Androgynous tassels are typical.
[6] Plants fail to grow.

growth appeared that is characteristic of blossoming plants was not greatly different in the several environments. Plants of *Nemesia* acquired an etiolated appearance at the warm temperature, especially in long days.

It has commonly been observed that corn does not make normal growth in the greenhouse at temperatures of 60° to 65° F.[5] At these

FIGURE 6.—Corn (Iowa Agricultural Experiment Station Selection No. 1445), which produced androgynous tassels at an intermediate temperature. Normal-appearing tassels and ears are produced at a warmer temperature.

temperatures plants in long days frequently produce androgynous tassels (fig. 6) and poor ears, and plants in short days develop abortive tassels but have more nearly normal-appearing ears. The variety Golden Glow as well as strains of sweet corn were very little affected by photoperiod at a warm temperature as normal tassels and ears were produced.

[5] ROBERTS, R. H., KRAUS, JAMES E., and LIVINGSTON, NORMAN, CARBON DIOXIDE EXCHANGE RHYTHM AND FRUITFULNESS IN PLANTS OF DIFFERENT REPRODUCTIVE HABITS. Jour. Agr. Res. 54 : 319-343, illus. 1937

EFFECT OF OTHER FACTORS ON THE RESPONSE OF PLANTS TO PHOTOPERIOD

There are a number of factors besides temperature which modify the effect of photoperiod on the growth of plants, as, for example, moisture and mineral nutrition. These, however, have not been varied in the present studies. Two other items have been given some attention, namely, the source of the plants and the effect of starting the light treatments at different periods in the life of the plants.

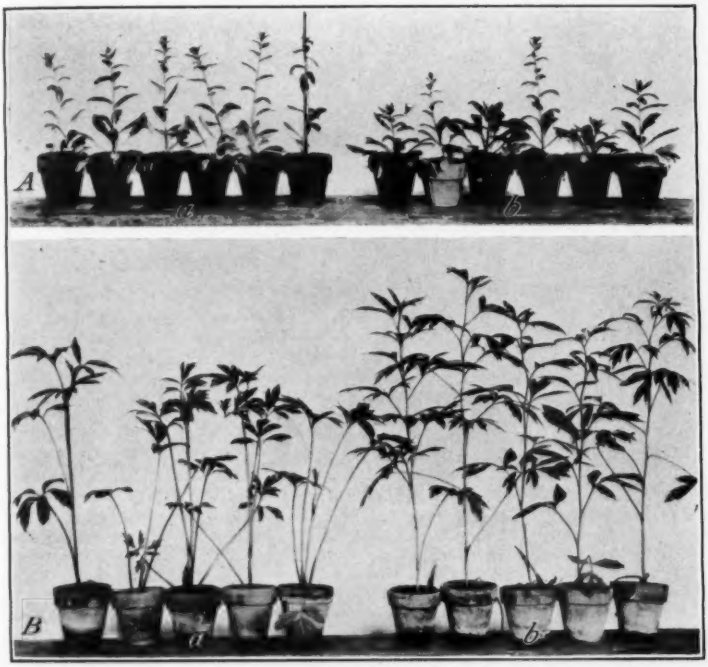

FIGURE 7.—Seedlings (*A*) or clons (*B*) may have a uniform or a variable population depending on the environment: *A*, Pennycress seedlings in cool, long-day (*a*) and cool, short-day (*b*) environments; *B*, *Rudbeckia laciniata* clons in cool, long-day (*a*) and warm, long-day (*b*) environments.

In another paper [6] it has been reported that plants grown from cuttings taken from a flowering plant made a different response to the same photoperiod treatments than seedlings of the same species. It was found also that cuttings from nonflowering plants reacted more nearly like seedlings than did cuttings from flowering plants. The time of reaction to photoperiod was also altered by the use of root-forming hormones. Cuttings of *Antirrhinum*, *Centaurea*, and *Petunia* were grown with and without a root-inducing "hormone." The use of commercial Auxilin according to the printed directions increased the vegetative growth but somewhat delayed the flowering of these clons.

[6] STRUCKMEYER, B. ESTHER, and ROBERTS, R. H. EFFECT OF PHOTOPERIOD AND TEMPERATURE UPON THE GROWTH OF SEEDLINGS AND CUTTINGS. In press. Amer. Jour. Bot.

Both clons and seedlings may be either variable or uniform depending on the environment in which they are grown (fig. 7). This variability can neither be predicted nor avoided in some locations. The production of a uniform population is obviously a matter of having a variety that is adapted to the treatment to be used.

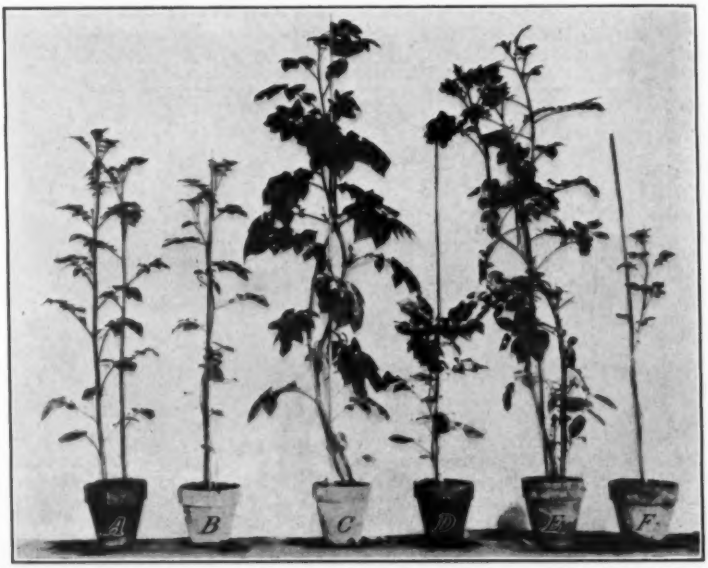

FIGURE 8.—The influence of the size of potato seed pieces on the growth of the plant is modified by the environment: *A*, Large piece, medium temperature, long-day; *B*, small piece, medium temperature, long-day; *C*, large piece, medium temperature, short-day; *D*, small piece, medium temperature, short-day; *E*, large piece, warm, long-day; *F*, small piece, warm, long-day. Like warm-temperature plants, the medium-temperature plants responded to photoperiod in short days, but like cool-temperature plants, made little response in long days.

Another instance of the effect of environment on photoperiodic influences was found in connection with the source of seed. When large seed-potato pieces (average 96.0 gm.) and small ones (average 7.0 gm.) were planted in warm long-day location, the plants from the large pieces were much more vigorous than those from the small pieces (fig. 8). This result was not consistent in all environments, however, as there was little effect of size of seed piece in a cool location. Similar results were secured in four tests.

The size of the corn kernel planted markedly effects the vigor of the seedling in a cool location, as the large kernels produce much larger plants than the small ones (fig. 9). In a warm location there is little influence from size of seed.

Another factor affecting the response which a plant makes to a photoperiod environment is, as Knott [7] has pointed out in the case of spinach, the stage of growth the plants are in at the time the treatment is begun. To secure the most desirable type of growth, many

[7] KNOTT, J. E. THE EFFECT OF TEMPERATURE ON THE PHOTOPERIODIC RESPONSE OF SPINACH. N. Y. (Cornell) Agr. Expt Sta. Mem. 218, 38 pp., illus. 1939.

FIGURE 9.—Large corn kernels produce stronger plants (*B*) than small kernels (*C*) when environment is cool, but no appreciable response is obtained when it is warm (*E*, *F*): Plants from medium-sized (*A*), large (*B*), and small (*C*) kernels planted out of doors in May; plants from medium-sized (*D*), large (*E*), and small (*F*) kernels planted in the warm greenhouse.

plants appear to require a change in environment as growth progresses. A clear illustration is that of blossom formation by alfalfa. Better clusters of blossoms were secured by starting the plants (of the strains used) at a cool temperature and then transferring them to an intermediate temperature (fig. 10, *B*). This change in environment proved more satisfactory in promoting flowering than keeping the plants continuously in either one of the environments. High night temperatures cause the blossoms to abscise (fig 10, *D*).

FIGURE 10.—A change in environment aids alfalfa blossom development (all long-day plants): *A*, Cool; *B*, cool, transferred to an intermediate temperature; *C*, intermediate temperature; *D*, warm (blossoms abscising.)

FIGURE 11.—Seed balls produced by Katahdin potato plants grown in cool, long-day environment but started in cool, short-day environment.

Good yields of seed balls of potatoes were produced by plants of the variety Katahdin which had come to an early blossom-bud stage in a cool short-day location and were then transferred to long days (fig. 11). Mature seed balls were not produced by the plants which remained continuously in the short- or the long-day situations.

DISCUSSION

The effect of temperature on the manner in which many plants responded to photoperiod reported here, together with the reactions

recorded last year,[8] appear to indicate clearly that photoperiod is not always the dominant factor inducing the blossoming of plants. Other cultural factors, such as seed and plant source and the condition of the plant when the treatment is begun, also indicate that the effect of environment is not constant. Photoperiod may be the primary factor for a certain range of temperature, but with many plants it is a contributing and not a controlling factor in the formation of flowers.

A uniform growth of a population may not be evidence that it is a homozygous one. The uniformity may be due merely to the fact that the environment did not induce an expression of the true heterozygous nature of the lot.

From a practical as well as an experimental standpoint, it would seem that more attention should be given to measuring the results secured when plants are shifted from one environment to another at different phases of their development. The most satisfactory growth of many varieties of plants cannot be expected by keeping them under a constant cultural environment throughout the entire period of their development.

SUMMARY

A large number of plants grown in long-day and short-day environments gave very unlike responses to photoperiod at different temperatures.

Corn plants grown in the greenhouse at warm temperatures were normal in appearance.

Plants grown from cuttings from flowering plants responded differently to photoperiod than did seedlings.

The variability of a plant population when grown from either seedlings or as clons was largely dependent on the environments in which the plants were grown.

The influence of size of seed on vigor of plant was affected by the environmental conditions under which the plants were grown.

A shift in the environment during the growth of many plants, as alfalfa, appears to aid in securing the most desirable type of development.

[8] See footnote 2.

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